

Etiquette for arms control

Politicians in the United States and the Soviet Union have a serious responsibility towards the arms control negotiations at Geneva — they must keep more quiet than is their habit.

THE best news from Geneva last week is that there was very little of it. Too many earlier conferences on arms control have been ruined by the recklessness of senior politicians on one side or the other in taking it into their heads to utter public statements about the course of the negotiations. Sometimes they have even advertised their "negotiating position" before letting the other side know privately what they have in mind. When people spill the beans like this, they often believe they are acting sensibly. The motive in going public is usually that such a step will strengthen the hands of negotiators operating behind closed doors, if only by reducing their freedom for manoeuvre. The difficulty is that successful negotiations demand a degree of flexibility, not to be mistaken for a readiness to compromise on issues of principle. In negotiations like that now begun at Geneva, the slow and inevitable leakage there will be about the conduct of the argument is immaterial. What will be damaging are statements made in public outside the negotiations which are intended to influence their course, or which declare that the outcome (whatever it may be) will be of no importance. Let us hope that there will now be a moratorium on public speaking.

Keeping quiet will be harder for the United States than for the Soviet Union. Constitutional pluralism can be a diplomatic embarrassment. But the United States is also caught up in several nasty almost domestic squabbles about strategic weapons, from Australia and New Zealand to Belgium (where the government's decision to let cruise missiles be installed has lent strength to the anti-nuclear movement). It will not help that the debate continues in the US Congress about the administration's authority to build another clutch of MX missiles. On past form, it is almost certain that some administration official will be tempted to go imprudently far, and in public. It is ironical that the dangers of the MX debate would be lessened if the United States were only to recognize that, even on military grounds, the MX is a mistake. It is too big and clumsy, and carries so many warheads that its deployment will entail putting too many eggs in too few baskets. (Each missile that fails on take-off will presumably have to be replaced by another, kept waiting in the wings.)

The Soviet Union, where plurality is not conspicuously a problem, is more strongly based. Mr Mikhail Gorbachev's accession may even help, by allowing subtle changes of policy seem less like concessions to pressure from outside. For what it is worth, the Soviet Union has, in any case, recently abated its earlier fierce complaint about the star wars issue. Condemnation of the idea persists, of course, and Soviet spokesmen keep saying there can be no agreement at Geneva without an agreement on the "demilitarization of space". That is legitimate enough, although the point has been made so often that it should now be repeated only in private (at Geneva). But the demand, loudly heard six months ago, that there should also be a moratorium on the testing of components of a space-based strategic defence has been quietly dropped. (A moratorium on the testing of anti-satellite weapons is a different issue, and would be valuable in its own right as well as an aid to a successful negotiation.) Temptations to speak out unwisely will nevertheless, no doubt, arise.

Meanwhile, both sides at Geneva are saying that the negotiations will take time, but that they could in the end succeed. That they take a longish view is a welcome sign of realism. But

it would have been more encouraging if the two sides had settled for a more empirical way of working, one that did not require that success should hang on a solution of all the world's problems at one stroke. For most people, a few modest steps towards a more comprehensive agreement would be preferable to the promise of an all-embracing agreement that could be snatched away by the simplest failure during the next few years. Conversely, a few modest agreements now — why not settle for ratifying the unratified treaties on the threshold test-ban and peaceful nuclear explosions? — would give the negotiators time to tilt at the big windmills.

The most obvious weakness of the arrangements on which the two sides at Geneva have agreed is that success hangs critically on success in each of three parallel sets of negotiations, on missiles of long and intermediate range and on star wars. The linkage between the first two issues is natural, given the strategic interest of Western Europe and other regions of the world, but star wars is logically separate, at least now. (Further ahead, if the United States had a working missile defence, the Soviet Union would be forced to build more missiles or to do something else.) The haunting difficulty is that if star wars had never been conceived, the missile negotiations would now be just as difficult as those that aborted at Geneva at the end of 1983. Is it not asking a lot, to hope to succeed now in an all-embracing negotiation when there was only failure to report in the simpler circumstances of eighteen months ago? After so much disappointment, why do the arms control diplomats allow themselves to be frustrated by the over-ambitious goals they set themselves?

To the errors of garrulity and over-expectation that repeatedly catch out the practitioners in this esoteric trade must be added a third — poverty of imagination. When arms control negotiations between the superpowers began, nearly thirty years ago, it was customary for the practitioners to try to visualize how things must seem to their opposite numbers, on the other side of the table. The view "from over the hill" was the standard phrase. That exercise seems to have lapsed. And both sides are to blame.

The Soviet Union, for example, has been inscrutably insensitive to the West's perception of its development in the strategic field. The large increase in the numbers of SS20 missiles (which have intermediate range) deployed in Western parts of the territory during the past ten years is shrugged off as if it were irrelevant to the strategic balance in central Europe. More recently, the phased-array radar that has popped up in Siberia, apparently in violation of the anti-ballistic missile treaty, went unexplained for more than a year and was then passed off as a component of a space-tracking network, as if there is nothing more to be said (or asked) about this installation. No doubt developments of a political character are even more unsettling of opinion over the hill. As things are, it must be assumed that the Soviet Union has given up trying to anticipate the impressions its actions cause.

The same, unfortunately, seems to be true of the United States. Nobody seems to have invited President Ronald Reagan to consider how the frequent claim that star wars (or authority for MX missile building) has forced the Soviet Union "back to the negotiating table" must seem inside the Soviet Union, habituated to the notion that the world is against it and now economically insecure as well. The claim, often heard, that the Soviet Union



has no business to react so angrily to star wars because its potential for making a first strike from the West feasible is a strictly academic matter, given that the United States would never do such a thing, must sound disingenuous in places such as Omsk and Novosibirsk. Even the assumption, commonly voiced in the United States, that the superior high technology of the United States (in the civilian field at least) will always ensure success in an arms race, may sound aggressive in the East. So should not Western politicians, like those elsewhere, learn not merely to hold their tongues for the duration of the Geneva negotiations, but also spend a little of the time they will save trying to see things from the other fellow's point of view? □

Hearts in the wrong place

The unauthorized use of an artificial heart in Arizona was mistaken humanity.

NATURAL sympathies are with the Arizona surgeon who two weeks ago commandeered an unapproved artificial heart in an effort, ultimately futile as it happened, to keep his patient alive while a replacement human heart could be found. The surgeon, Dr Jack Copeland, has been cast in the traditional American role of the rugged individualist defying authority to do what is right. The Food and Drug Administration (FDA) has obligingly filled in with the part of the pointy-headed bureaucrat by mumbling about technical violations of regulations.

The facts of the case are these: a 33-year-old man who had had two heart attacks and a poor chance of living much longer was given a new human heart in what is becoming an increasingly routine operation. The new heart, however, stopped beating the next day. While Dr Copeland began the search for a replacement, the patient was placed on a heart-lung machine. As the hours passed — and a patient can be kept on the heart-lung machine only a few hours before his red blood cells begin to be damaged — Dr Copeland decided the only hope for his patient was the temporary implantation of a mechanical heart.

FDA had authorized only one surgeon — Dr William DeVries of the Humana Heart Institute in Kentucky — to perform that procedure, and only with the Jarvik-7 heart, which was developed at the University of Utah. For reasons that are still unclear, Dr Copeland called St Luke's Hospital in Phoenix, and asked surgeons to bring a mechanical heart under development there. That heart, developed by a dentist, had not been submitted to FDA for approval. It had never been used in a human. Its longest test had been in a calf, for 12 hours. (The Jarvik heart was tested for months in a calf before the first clinical test.) A Jarvik-7 heart was flown in as well, but was not used; some reports say the University of Utah decided against participating in the unapproved surgery.

The Phoenix heart kept Dr Copeland's patient alive for 121 hours; a new human heart was then transplanted but by then lung damage had taken its toll and the strain on the new heart was too much. The patient died, three days after the ordeal began.

Dr Copeland and his surgical team say, simply, that there are higher laws than FDA when a patient's life is at stake. Yet the very point of FDA's regulatory role is to prevent the sort of haphazard and unplanned experiments on humans that in fact took place here. Informed consent — which was obtained from the patient's relatives — is only one necessary element in assuring the ethical practice of experimental medicine. In a crisis, what relative is going to say no to a doctor, especially one so obviously sincere as Dr Copeland in wanting to do the best for his patient? It may be a cruel thing to say, but the end result of what happened in Arizona is that a human being was used as a guinea pig. The FDA rules, hardly extreme, are designed to assure a minimum degree of safety and likely benefit before humans become the experimental subjects.

The irony of the entire episode is that, with better planning, what was attempted at Arizona is really the way that artificial hearts ought to be put to use now: as a stop-gap during heart-transplant surgery. After the Arizona episode, FDA did in fact

approve yet another artificial heart — this one developed in Pennsylvania — for just this purpose. Heart transplants can now offer real hope for otherwise hopeless patients. They have also succeeded in many cases in allowing patients to resume near-normal, active lives. The same simply cannot be said for the "permanent" implantation of artificial hearts at this primitive stage of development. An implant that must be tethered to a huge external air compressor (apart from posing a constant risk of infection at the point where the tubes enter the patient) exacts a devastating psychological toll.

The argument, of course, is that the early heart transplant operations could have been criticized on similar grounds — that they were grandstand medicine, more stunts than attempts to improve the patients' well-being — but that what was learned in these many early attempts paved the way for today's successes. The analogy is not exact, however. The medical spectaculars at the Humana Heart Institute (which has permission from FDA to go ahead with more "permanent" artificial heart implantations) are turning out to be gruesome endurance tests. No matter how much is learned about surgical technique, proper medication and the like from these operations, the technology of the heart itself is simply too primitive to justify the goal of permanent implantation. They are attempting to fine-tune a procedure that is questionable even if it worked perfectly.

Admittedly, clinical performance data need to be collected hand-in-hand with efforts to improve the technology. But limiting the artificial heart to temporary use seems a reasonable compromise to achieve this goal without turning humans into guinea pigs. □

Sustaining Africa

The UN now has money with which to feed Africa. How should it be spent?

THE United Nations has surprised itself by collecting (in promises) more than twice the \$1,000 million it had asked for so as to reinvigorate African agriculture. No doubt part of the success stems from this season's appalling famine in Ethiopia, worse than last season's by a long way if only because it was more elaborately covered by television cameras. Unfortunately, it is far from easy to tell how such large resources could be spent wisely and quickly enough to make much difference within the lifetime of those who have worked to recruit the funds. The temptation will be to spend the money on the classical agricultural projects of development — irrigation dams and drainage schemes. But the needs are social and even political.

Ironically, most industrialized countries honour their farmers and are indeed over-generous towards them, but developing countries, where the need for food is greater, tend to worry instead about the prices that urban people (and voters) pay for the food they eat. The result is price control that pauperizes the farming population, driving them to the cities to look for welfare instead of growing food. On many occasions, matters have been made worse by spectacular mismanagement, as of Ghana's cocoa crop, once the foundation of the country's relative prosperity. In Ethiopia, part of the cause of this year's famine must be the hostility of the government (and, fair play, of the farmers) towards the middlemen who traditionally bought grain from farms and sold it on to cities; nobody remembered that the middlemen were the distribution system, which collapsed when the intermediaries were abolished. There are also, of course, climatic and agricultural problems of great severity.

The most urgent need, and the best use of the UN money, is for a technical infrastructure to support peasant agriculture, Africa's equivalent of the extension services of Europe and North America (but also in place in India before the green revolution). The second need is for better plant breeding, done in Africa (but not necessarily by Africans.) The third is that African governments should recognize that the whole country will go hungry if farmers are driven to starvation by low prices. There might even be some change left over for a few dams. □

Genetic engineering patents

Contest in prospect over interferon rights

Washington

BATTLE lines are now being drawn up in what may be the first major contest for patent rights over a commercially significant product of genetic engineering.

The product, human leukocyte (alpha) interferon, has shown potential in clinical trials against some cancers and viral diseases, including the common cold. Last year Biogen NV of Cambridge, Massachusetts, and Geneva, Switzerland, announced it had received a European product patent for alpha interferons produced by recombinant DNA techniques; the company expects shortly to be issued with a US patent covering the same claims. Now, however, Hoffman-LaRoche Inc. of Nutley, New Jersey, has announced that it has received a US patent covering "all highly pure human leukocyte interferons no matter how they are made". John Saxe, chief patent counsel for Roche, says the company is "not aware of any highly pure leukocyte interferon product that does not fall within the issued claims". Schering-Plough Corporation, which produces Biogen's interferon (trademarked Intron) under licence, immediately challenged Saxe's claim, denying that Intron fell within the scope of Roche's patent.

The Roche patent is based on the 1970s work of Sidney Pestka and Menachem Rubinstein, in which they isolated and purified alpha interferons from human cells. LaRoche expects to receive a further US patent covering its own recombinant alpha interferon, Roferon-A, which was developed in collaboration with Genentech Inc. of San Francisco. The patent already issued to Roche defines interferon by its anticytopathic activity as assayed by two standard cell lines but does not include sequence data; Biogen's European patent, in contrast, does specify the product by sequence. A further complication is that Biogen's patent, which was filed before that of Genentech/Roche, strictly refers to an interferon precursor molecule, not the mature molecule, according to Genentech.

Alpha interferon is undergoing clinical trials at several centres in the United States. Diseases in which it has shown potential include Kaposi's sarcoma (often associated with acquired immune deficiency syndrome), multiple myeloma, malignant melanoma and hairy cell leukaemia. Both Schering-Plough and Roche have submitted marketing applications to the Food and Drug Administration for their respective products, which differ by a single amino-acid residue. Schering's Intron is approved for use in two indications in Ireland and the Philippines.

The courts have yet to test patent law as applied to genetic engineering products, and many small companies are seriously concerned that their product options might be constrained by broad product patents. Both Schering-Plough and Roche, as major corporations, have the resources to endure a court battle should one become necessary: Peter Feinstein of Biogen says he is confident that Schering will be able to prevent Roche from marketing Roferon-A. But industry analysts point out that large

pharmaceutical corporations tend to avoid court cases wherever possible, and some sort of compromise may yet be negotiated.

Whatever the resolution of the present dispute, it is unlikely to serve as a model for future cases; with each product patent issued, the extent of "prior art" increases and future patents become harder to defend. One possible pointer to the future, however, is the recent US patent granted to Cetus Corporation covering what it calls muteins of interleukin-2, a lymphokine with anticancer and antiviral potential. The muteins are specific modifications of the natural protein: the Cetus mutein is claimed to be more stable than natural interleukin-2. Just as important, however, Cetus is confident that its mutein will lie outside the scope of other interleukin-2 patents.

Tim Beardsley

Military manoeuvres

Scientists' Nazi past laundered

Washington

US ARMY intelligence officers deliberately altered the dossiers of German scientists brought to the United States at the end of the Second World War to conceal their Nazi party activities and, in some cases, even their indictments for war crimes. Government documents released for the first time leave little doubt that the Army's intention was to circumvent President Truman's order that no "active supporter of Nazism or militarism" be allowed into the country under the programme of recruiting German "specialists".

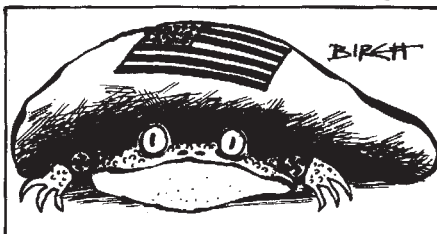
Among those whose dossiers were altered was Wernher von Braun, the rocket scientist who later assumed a leading role

"ardent Nazis". The director of the recruitment programme, Bosquet Wev, then complained to the Army's intelligence director that "the best interests of the United States have been subjugated to the efforts expended in 'beating a dead Nazi horse'". Wev said "the most positive and drastic action" was needed. The most revealing document came later that year: Wev again wrote to Army intelligence, and enclosed dossiers of 14 scientists, including von Braun: "... security reports recently forwarded from your headquarters classify 14 specialists as potential or actual threats to the security of the United States... there is very little possibility that the State and Justice Departments will agree to immigrate any specialist who has been classified as a... security threat... It is requested that the cases of the specialists listed in paragraph one be reviewed and that new security reports be submitted where such action is deemed appropriate in view of the information submitted in this letter." All were subsequently changed.

Other dossiers — such as those of the scientists indicted at Nuremberg — appear to have been "clean" from the start. The dossier of Arthur Rudolph, the NASA scientist who left the United States last year to avoid being deported for his role in the persecution of slave labourers at the V-2 rocket plant that he managed during the war, made no mention of evidence from the Nuremberg trials implicating Rudolph nor of his having joined the Nazi party in 1931.

All told, 765 scientists and technicians were recruited under the programme. The documents confirm that a major concern of the US officials who ran the programme was not letting the German scientists fall into Soviet hands. Wev wrote in one memorandum: "The return of these scientists to Germany would present a far greater security threat to the United States than their retention in this country".

Stephen Budiansky



in the US space programme. Von Braun's original dossier labelled him a "potential security threat".

The Army also attempted to recruit four scientists who were later indicted at Nuremberg for war crimes. One was sentenced to 20 years' imprisonment for participating in experiments in which concentration camp inmates were forced to drink sea water.

The documents, which are the first to show a deliberate cover-up by the Army, were obtained by a reporter for the *Bulletin of the Atomic Scientists* under the Freedom of Information Act. A full account will appear in the April issue of the *Bulletin*.

Under President Truman's order, the State Department had to pass on scientists recruited by the Army. In early 1947, the State Department refused visas for several scientists labelled in security reports as

Anthropology

Genetic links for scattered Jews

Rehovot

PRELIMINARY studies using DNA sequences as a new and sophisticated tool for genetic analysis tend to support the conclusion drawn from earlier investigations that the Jews, even after being scattered around the world for two millennia, remain — to a significant degree — genetically distinctive.

This view, propounded for many years by Professor Batsheva Bonné-Tamir from the Department of Human Genetics at Tel Aviv University's Sackler School of Medicine, has met with opposition from some other scientists who use different statistical methods and from those who feel that any attempt to suggest the existence of a specific Jewish group is to be rejected as racist doctrine. Professor Bonné-Tamir is obviously not a proponent of such a doctrine, nor does she suggest that the Jews are better or worse for having a common genetic heritage. She simply points to the evidence that it exists.

Among the genetic markers used in the past by Professor Bonné-Tamir are blood types, enzymes, serum proteins and histocompatibility antigens (see *American Journal of Human Genetics* 34, 50; 1982). The new data, which have been submitted for publication, come from the most direct genetic measure — that of DNA sequences, in this case of short stretches of mitochondrial DNA. But whatever the criterion, she and Professor Samuel Karlin of Stanford University in the United States find that Jews from Iraq, for example, have far more in common from a genetic viewpoint with Jews from Poland than either group has with the non-Jews among whom they have lived for centuries. This is also true of immigrants to Israel from such diverse areas as Germany and the Soviet Union on the one hand and Libya on the other.

It has become possible to make more revealing comparisons between Jews and non-Jews in recent years because of the increasing number of genetic studies on both.

There are, it seems, a few exceptions to the general picture of relative Jewish distinctiveness. Jews from Yemen, often popularly regarded as the Jewish group that looks most "biblical", are a case in point. "They have a genetic make-up", Bonné-Tamir reports, "that is characteristic of the Saudi-Arabian peninsula and are probably the descendants of indigenous tribes which converted to Judaism in the fourth and fifth centuries."

A similar situation prevails in regard to Cochin Jews and the local Kerala Indians, communities that are genetically linked to one another but have little in common, from a genetic viewpoint, with other Oriental-Jewish groups or with non-Jewish elements in southwest Asia. This fits in well, Professor Bonné-Tamir points out, with the theory proposed by Professor

Avinoam Adam of Everyman's University (Israel's equivalent of the UK Open University), who has written: "in some areas and during some specific periods, the Jewish religion expanded much faster than Jewish genes".

Most timely of all, in view of the present wave of immigration from Ethiopia, are her findings about the Jews from that country. Various theories exist as to their origins, with members of the community itself tending to argue that they are descen-

dants of the tribe of Dan (one of the original 12 tribes of the Bible) and/or of ancient Jewish communities in Upper Egypt that moved south. Yet after studying representatives of a hundred immigrant families from Ethiopia, Professor Bonné-Tamir comes to the conclusion that genetically they most resemble the Amhara tribe, and to a lesser extent the Billen and Tigre tribes, of Ethiopia. But she says "there is no denying the fact that the Yemenite, Cochin and Ethiopian Jews have long displayed strong emotional bonds with Judaism and rushed to join their co-religionists in Israel as soon as they had the opportunity to do so."

Nechemia Meyers

Australian technology

OECD begs leave to differ

Canberra

AS soon as the Organization for Economic Cooperation and Development (OECD) draft report on the state of Australian science and technology was made available last month, it became obvious that many of the recommendations for improvement were exactly the opposite of those in operation at present or likely in the near future. The examiners asserted that it was essential that the science and technology portfolios be combined and represented at Cabinet level by a strong minister. An Australian federal election was held between the first draft of the report and its submission to the Australian authorities, the upshot of which was a portfolio reshuffle, splitting Mr Barry Jones's Department of Science and Technology, with politically important policy sections being absorbed by Senator John Button's Department of Industry and Commerce (see *Nature* 5 January, p.4). Mr Jones's remaining Department of Science has in the meantime been casting about for new vistas in science without technology, a condition described by the examiners as "perverse", because, in their view, the topics are so completely intertwined. The government's action also puts paid to the examiners' recommendation that the Australian Science and Technology Council (ASTEC) should report to a "strong science minister" rather than supply policy advice directly to the Prime Minister, which has been its brief since 1976.

The difficulty is the notion of a single strong minister for science and technology. Senator Button certainly fills the bill for his now-extended Department of Industry, Technology and Commerce, but part of the reason for the political downgrading of Mr Jones's portfolio was his unwillingness to linger in smoke-filled rooms where the deals are done. It is difficult for citizens of nations where science and technology are not (yet) an issue to appreciate the job of advocacy that Mr Jones has done in Australia, and the void that existed before him. The examiners noted that many of the depositions submitted to them in the course

of the study bore witness to the discussions he had sparked in 1983-84, but something else shone through as well: a sense that science and technology were somehow external to national life and that the transition from research to design and sales of a product seemed to involve the collision of mutually uncomprehending cultures.

Part of this they attributed to the education system and recommended (*inter alia*) that research in universities and other tertiary institutions be strengthened, especially by the purchase of modern equipment, both propositions unlikely in the short run, given the Treasury's present parsimonious mood. The examiners supported some tactics — such as manpower planning — that are unlikely to find favour, and suggested that Australia should not follow blindly the example of other countries in developing "key technologies" such as information technology and biotechnology, but instead examine its own latent strengths on a sectoral basis, suggesting pharmaceuticals as a possible new industry. Whereas in the past Australia has used bounties and tariffs to shore up industry rather than the system of fiscal incentives common in other OECD countries which encourages business enterprise to classify its activities as research and development for tax purposes, the examiners recommended that tax-based schemes be instituted in Australia, a strategy the government has already begun and may expand.

Although preceded by an OECD *rapporteur* a year ago, the examiners themselves spent only one week in Australia. Inevitably, their report must be coloured by views expressed in the submissions made to them. They seem awake, nonetheless, to the pressures that have been brought to bear on OECD referees in the past, noting that familiar arguments about the economic benefits of investment in intellectual and technological infrastructure may be needed for the benefit of the Commonwealth Department of Finance. A final consultation and draft are due later this year.

Jeffrey Sellar

Soviet policy

Gorbachev on science

SOVIET scientists, and, indeed, Soviet society at large, must pay greater attention to the challenge of modern technology, according to the new General Secretary of the Communist Party of the Soviet Union, Mikhail Gorbachev. Addressing the Central Committee last December, in place of the ailing Konstantin Chernenko, Gorbachev noted that although in many enterprises and scientific institutions research and development problems are resolved "up to the standards of the world's top achievements", many "collectives" are still satisfied with repeating what has been outstripped in world practice.

Paramount importance, Gorbachev told the Central Committee, must be attached to the strategy for developing scientific, technical and production potential, and, in particular, to "fundamentally new and really revolutionary scientific and technical solutions" which will increase labour productivity many times over.

This has been standard party policy for some time; what is, perhaps, significant is Gorbachev's emphasis on the idea that scientific and technological progress can be achieved only by raising the "cultural and technical standard" of the working class and peasantry, and "a radical improvement in the training and perfecting of society's main productive force". This, he said, will entail a major restructuring of the "cadre training system" at all levels of education from primary school up to university. This work is already under way, he noted, but "must acquire a large-scale character".

Educational reform, in particular the changes introduced in September 1984, is apparently a subject in which Gorbachev takes a special interest. One of his last major engagements before his elevation to the general secretaryship was to chair a meeting of the Commission on the Reform of General and Vocational Education. The 1984 reforms stress "practical labour training, and vocational orientation" for all school-children, and the appropriate production ministries are expected to help provide necessary facilities. The February meeting of the commission, however, found that the All-Union Ministry of Light Industry and a number of light industry ministries in the union republics have in many cases contented themselves with drawing up "plans for measures and instructions". Local government officials and education committees, likewise, have been doing little to implement the reform.

This slackness, the commission decided, will not do. Within the next two to three years, it warned, all school-children must be properly involved in socially useful and productive labour, and at the same time, all secondary-school pupils must be computer-literate.

Vera Rich

Bendectin/Debendox

Drug not guilty, says court

Washington

A FEDERAL jury last week decided that there is no evidence to link Bendectin, an anti-nausea drug used in pregnancy, and birth defects. The court, in Cincinnati, Ohio, dismissed a case brought by 1,100 women across the country, representing two-thirds of outstanding US claims against Merrel Dow pharmaceuticals, the maker of the drug.

A spokesman for the company said the decision established that a product liability case could be successfully defended in the United States and indicated that Merrel-Dow will now be in no mood to propose no-liability settlements in the remaining cases. Earlier, the company had offered \$120 million in settlement of all outstanding and future claims.

Bendectin (known in the United Kingdom as Debendox) was available as a prescription drug in the United States from 1956 to 1983, when it was withdrawn by the company because of high insurance premiums resulting from the accumulating claims and the bad publicity. Merrel Dow says that no fewer than 27 epidemiological studies have failed to turn up any evidence that the drug causes birth defects. The active ingredients are doxylamine (an anti-

histamine) and vitamin B6. Before 1976, Bendectin also contained dicyclamine, which was removed because of insufficient evidence that it added to efficacy.

The Cincinnati ruling is the third courtroom vindication for Merrel Dow. A case in Florida in 1981 was decided in the company's favour; in 1983 a jury in Washington DC awarded one plaintiff \$750,000 damages but was overruled by the trial judge. The publicity made Bendectin a potential bonanza for tort lawyers, some of whom started to advertise for parents of children with birth defects who might have been affected by Bendectin.

Recognizing that the mass of litigation threatened to clog the legal system, the trial judge in the most recent case took the innovative step of holding a common issues trial to decide questions relevant to all the 1,100 plaintiffs. In an attempt to avoid having to face future litigation on Bendectin, Merrel Dow offered \$120 million as a class settlement to cover all future claims. The offer was accepted by the majority of attorneys in the case but was challenged by some dissidents; the class settlement was then disallowed by an appeal court on a technicality, necessitating the trial that ended last week.

Tim Beardsley

Soviet Union

More nuclear research

THE Dubna Joint Nuclear Research Institute in the Soviet Union is to expand its area of research considerably during the coming five-year plan (1986-1990), its director, Academician Nikolai N. Bogolyubov, announced last month. Addressing a meeting of government plenipotentiaries of the 11 member states of the institute (the 10 Comecon partners plus North Korea), Bogolyubov noted that during the next five-year plan "great attention" would be paid to identifying applied work, using the results of fundamental research in related spheres of science and technology, in particular medicine, geology, biology, metallurgy and ecology.

Applicability of fundamental work is a keystone of Soviet science policy, and in a formal keynote speech need not have anything other than a purely ritual significance. Bogolyubov, moreover, has a background in fundamental mathematics and physics and has just been awarded the Lomonosov medal, the highest honour of the Soviet Academy of Sciences, for his theoretical work. Nevertheless, at a time when Comecon research plans in all fields are being closely coordinated, the commitment of Dubna to finding new applications for its research probably represents a genuine statement of intent, especially for the less prosperous member countries.

In his speech, Bogolyubov put con-

siderable emphasis on the "powerful experimental base" that Dubna has acquired in recent years, including the "unique" pulsed neutron fast reactor which came into operation last year, and the plans for the construction of a nucleotron — a superconducting accelerator with the aid of which, he claimed, it will be possible to accelerate nuclei of virtually all the elements in the Mendelev table, right up to uranium. Dubna's latest achievement, announced shortly before the February summit, also lies in the theoretical field: the creation of element 108. According to the official announcements, in the spring of 1984, in work on the new U-400 cyclotron at Dubna, 44 cases of the decay of three isotopes of element 108 were recorded.

In spite of the "experimental base" praised by Academician Bogolyubov, however, not all is well at Dubna. The central computer centre, in particular, seems to be inadequate, judging from the stress placed on the need to enlarge its capacity. The "instrument centres" of the various laboratories, too, need to be expanded.

● The Soviet Union, of course, has other atomic research institutes. Foremost among these is the Kurchatov Institute of Atomic Energy in Moscow which, says Academician Evgenii Velikhov, has a considerable lead over the West in fusion research.

Vera Rich

CERN

How to pay British share

BRITISH Foreign Office subscriptions to international bodies are effectively protected against exchange rate fluctuations by the Treasury — so why should not the international subscriptions of the Science and Engineering Council (SERC) be similarly protected? Two Oxford high-energy physicists, Professor Don Perkins and Dr John Mulvey, have put this question to their local Member of Parliament, Tory John Patten, who has promised to raise the matter with Sir Keith Joseph (Secretary of State for Education and Science). Sir Keith is still awaiting news from the Kendrew committee on Britain's future role in high-energy physics, so the physicists' intervention is opportune. Friction between high-energy physicists and others supported by SERC, which pays the annual subscription to CERN (the European Organisation for Nuclear Research), is exacerbated by the fluctuating cost of the subscription in pounds sterling against what is now a constant subscription in Swiss francs, and any realistic way of handling the fluctuations would at least ease tempers.

According to Perkins and Mulvey, the issue is this. Member states of CERN pay their subscriptions in Swiss francs according to their "net national income" (NNI) averaged over three years. The proportions are determined every three years using the most recent statistics of the United Nations Statistical Office. But these statistics can be three years old, with the result that actual payments can be six years out of line with real current NNI. "For example, the present shares were decided in 1983, using data for 1979-81. They will be in force for 1984-86."

Nevertheless, in the long run, exchange rate fluctuations are automatically corrected, as NNIs are calculated in dollars. So if sterling falls the British NNI falls with it. But the delay in calculations causes trouble. Recently, the sterling cost of the CERN subscriptions has risen sharply from £23 million in 1981 to £35 million in 1984 because sterling, and hence NNI, was strong against the dollar in 1979-81 but has fallen nearly twofold since.

According to Perkins' and Mulvey's calculations, the net overspend (out-turn minus estimate) by SERC on the CERN subscription over the eleven years starting 1971-72 was only £4.75 million (just over one per cent of the total). From year to year, however, the overspend has been as high as £4.5 million (1976-77) and as low as minus £8.7 million (1980-81) — a profit for SERC.

"Exchange rate and relative NNI changes are quite outside the control of anyone", write Perkins and Mulvey, "and the application of 'cash limits' (inclusive of the international subscriptions) to the already desperately strained SERC budget does not encourage economy. It only sub-

jects the scientific programmes to arbitrary jolts which have very damaging, and wasteful, effects."

Thus they recommend:

- That subscriptions to international research organizations (such as CERN) should remain part of the budget of the appropriate scientific policy-making body (for example SERC). "Then the trend of the budget... can be decided in the right scientific context."

- That the delay in the calculation of relative contributions be shortened, "though this may not be welcomed by countries with more stable currencies".

- That SERC could pay foreseen subscriptions in sterling, while the Treasury corrects for changes in the exchange rate and NNI by adding or subtracting the appropriate sums. "This separates the functions of making scientific policy (SERC) and solving the technical problems (Treasury)... Experience shows that the Treasury (and SERC) would not lose (or gain) over a number of years."

Moreover, Perkins and Mulvey claim, "there can be no objection in principle to such a mechanism since cash limits are not applied to the international payments made by the Foreign and Commonwealth Office".

Here, however, there appears to be some uncertainty. According to a Treasury spokesman, Foreign and Commonwealth Office (FCO) international subscriptions (to the United Nations, for example) are indeed not cash limited, which means that the Treasury will bail out FCO if there are political changes in those subscriptions during a financial year, but on currency fluctuations, FCO must look for "offsetting savings", according to the Treasury. However, unlike SERC, FCO has a long-standing "arrangement" with the Treasury by which the fluctuations are discussed as they occur.

Robert Walgate

NSF deputy at last

DR John H. Moore, an economist at the Hoover Institution in Stanford, California, has been nominated by President Reagan to be deputy director of the National Science Foundation (NSF), a post that has been vacant for two years.

Although Dr Moore has an undergraduate degree in chemical engineering and worked as an industrial research chemist for four years, his PhD is in economics, a subject to which he has devoted the bulk of his professional career.

Moore has been a member of the National Science Board, NSF's formal governing body, for the past two years.

The Hoover Institution is a generally conservative think-tank associated with Stanford University. □

Archaeopteryx

Fraudulent feathers?

SCIENTISTS at the British Museum (Natural History) met on Monday to launch their response to the mounting publicity surrounding the claim by Lee Spetner in Rehovot and a group including Fred Hoyle and Chandra Wickramasinghe from University College Cardiff, that the feather impressions on their specimen of the famous fossil bird-like reptile *Archaeopteryx* may be a fake.

The controversial claim has been raised by an editorial and an article accompanying new photographs of this specimen published by the group in the *British Journal of Photography* two weeks ago. The crux of the claim is that many of the feather impressions occur on patches of material that are much finer-grained than the underlying rock and in parts the material looks like "flattened blobs of chewing gum". The possibility is that this represents some sort of cement that was applied to the fossil after discovery, on which feather impressions were made.

To elevate the argument from rhetoric and refutation, the scientists at the museum decided at their meeting to carry out two tests on the fossil specifically to address the possible existence of a cement layer. They will first take a section of material from the edge of the specimen for standard microscopic sedimentational analysis to detect any differences in particle size and the existence of a boundary between a surface layer and underlying material. They will then use electron microprobe analysis to compare X-ray spectra emitted by surface and underlying material.

The team is not, however, optimistic that these tests will provide clear-cut evidence and is already aware that a cement made from underlying rock might not be detected by these analyses. Although ready to carry out these tests immediately, the team feels it may be best if the areas selected for analysis are chosen by Hoyle's group.

The decision to allow samples of material to be taken from this extremely important fossil results from the conviction, voiced by Dr Alan Chong of the Department of the Palaeontology at the museum, that the specimen is genuine. He would seem to have considerable support from the existence of five other specimens assigned to *Archaeopteryx* from the Upper Jurassic. All have associated feather impressions but were discovered at different times and at different sites. Indeed, the incomplete Tyler specimen discovered in 1855 was first described as a pterodactyl, and it was not until 1972 that John Ostrom noted the feather impressions and described it as *Archaeopteryx*. If the feather impressions are the work of a single faker, he must have been remarkably fleet of foot and very prescient.

Nigel Williams

Genetic diseases

Problems of prenatal testing

Washington

NEW techniques for prenatal diagnosis of genetic diseases are sharpening the debate in the United States over when the Food and Drug Administration (FDA) should approve for commercial use test kits that might lead a woman to prefer an abortion to the possibility of bearing a handicapped child. Methods based on analysis of fetal DNA promise to increase greatly the number of diseases for which prenatal diagnosis is possible, but the anti-abortion lobby is firmly opposed to any test used in a "search and destroy" mission. And the anti-abortion lobby cannot be dismissed as part of the lunatic fringe: President Reagan has made clear his opposition to abortion under any circumstances.

Prenatal testing for some easy-to-detect conditions such as Down's syndrome and Tay-Sachs disease has been carried out for several years. Although in some instances a good argument can be made that tests have increased, rather than decreased, births of healthy but potentially affected children, the strength of anti-abortion sentiment suggests that the arguments will have to be fought anew as each new test becomes available. Furthermore, the spread of chorionic villus testing, which allows fetal cells to be obtained earlier in pregnancy than by amniocentesis, could make easier those abortion decisions based on tests for inherited conditions: the National Right to Life group is already challenging use of the technique.

FDA spokesmen are wary of speculating about the regulatory passage of future commercial kits, but it is clear that any company wishing to market a new prenatal diagnostic will have to go to unusual lengths to provide data on false positive rates before approval can be expected; a false positive result could lead to the unnecessary abortion of a healthy fetus. Industry spokesmen hope that FDA will restrict itself to questions of safety and efficacy, disregarding the thorny risk/benefit issue, with its attendant questions about the quality of life of a handicapped child. But some recent FDA decisions — on putative cancer therapies, for example — suggest that FDA does indeed consider it part of its job to scrutinize possible benefits as well as safety.

In 1983, after seven years of foot-dragging, FDA eventually approved unrestricted use of test kits to detect elevated concentrations of α -fetoprotein (AFP) in amniotic fluid or serum, indicative of neural tube defects such as spina bifida. That decision reversed FDA's earlier position, in which it had sought to specify the testing protocol and interpretation of results for AFP kits. Although it is pointed out in defence of FDA's delay that there are good reasons to be wary of uncritical use of AFP tests, few tests would claim to

be 100 per cent efficient — in any case, numbers matter little to those whose opposition to abortion is based on religious grounds; it remains to be seen whether future tests will experience similar delays.

Many prenatal diagnostic tests, such as those for sickle cell anaemia and phenylketonuria, are now being performed in university medical centre research programmes, approved by institutional review boards but not by FDA for unrestricted use. Commercial reference laboratories, in contrast, use only FDA-approved reagents. The limits of FDA's authority to regulate experimental tests are "somewhat cloudy", according to an official in FDA's compliance division: although there is no question that an experimental test can be used for evaluation in conjunction with an established technique, or if it presents no significant risk, established tests are sometimes lacking, and abortion decisions are being taken on the basis of experimental tests. There is at least one published case of an abortion resulting from incorrect experimental diagnosis of a haemoglobinopathy.

For some of the genetic conditions that can now potentially be diagnosed by

analysis of linked fetal DNA markers, such as retinitis pigmentosa, Duchenne muscular dystrophy and Huntington's disease, testing of relatives is necessary (but not always sufficient) to arrive at probabilistic estimates that a given fetus is affected by an inherited disorder. As long as this sort of specialized knowledge is required for testing, work will be restricted to experimental medical centres and so remain effectively beyond FDA's purview, even though such testing is offered as a commercial service.

But the goal of much current research is to produce probes that will identify a mutant gene directly, or, even better, the gene product. Recently, for example, a direct gene probe for prenatal detection of phenylketonuria has been obtained. When simpler tests become available error rates will fall, and commercial kits will be feasible. One of the greatest hopes is for a test for cystic fibrosis, the commonest lethal genetic disease in the United States. The gene responsible for the disease has not yet been identified, but development of a test is "at an advanced stage", according to Integrated Genetics Inc. of Massachusetts. Although the eventual FDA approval for AFP test kits is hailed as a precedent for future tests, the industry knows that it will have to work harder in future to satisfy FDA demands. **Tim Beardsley**

Geology

New Chinese institute

A NEW Chinese research institute, the Xian Laboratory of Loess and Quaternary Geology (XLLQG), has been set up in Xian, Shaanxi Province. The laboratory is an independent unit, attached to Academia Sinica, and is engaged in the study of the characteristics and formation processes of loess and other Quaternary sediments in China. On the basis of the geological and biological records of the Quaternary period, the workers at the institute will try to reconstruct the history of the environmental changes of the Quaternary, and to understand the effect of human activities on future developments. This reconstruction of the pattern of environmental change is the single most important task facing Quaternary investigators, and those involved with XLLQG will be well placed to make significant contributions.

Xian is in the middle reaches of the Yellow River with the great Loess Plateau and widespread deserts to the north and west. To the east, Xian faces the North China Plain, and to the south, across the Qin Ling mountain, is the Red Basin of Sechuan Province with temperate and subtropical landscapes. The continuous loess-palaeosol series which began to be deposited 2.4 million years ago (see *Nature* 300, 431; 1982) provides one of the best available indicators of Quaternary climatic changes, and contains abundant traces of early humans and Quaternary vertebrates.

Several research projects are already under way; these include investigations on the composition, physical characteristics and micromorphology of loesses and palaeosols and their relation to environmental changes and engineering construction; processes and mechanisms of recent dust falls and loess sedimentation and the relation of loess accumulation and loess erosion; the evolutionary history of human fossils on the Loess Plateau and the migration of fauna and flora during the Quaternary; loess-palaeosol sequences and their meaning to palaeoclimate since 2.4 Myr BP, and the sequence pattern and its development in northern China; and the recent climatic change (over about the past 2,000 years) and its relevance to human activities on the Loess Plateau and in northern China.

The first director of the new institute is Liu Tungsheng, with An Zhisheng as deputy director. Liu says he expects XLLQG to be a laboratory of international character. It is hoped that scientific and technical cooperation between China and other countries will not be limited to the exchange of research workers, but will also include joint ventures. This idea is fully supported by Academia Sinica and the new institute should provide a chance for investigators from all over the world to work in one of the really classic loess regions. **Ian Smalley**

Radiation data wanted

SIR — In 1975, the International Commission on Radiological Protection (ICRP) published its Publication 23, the Report of the Task Group on Reference Man. Publication 23 is about 15 years out of date, because the references cited end around 1970. At its 1984 meeting, ICRP approved the formation of a new task group to revise and update this publication, with completion expected in about three years.

The revision will include more emphasis on variations due to age, sex and other individual differences in anatomical and physiological data and in the gross and elemental composition of tissues. The emphasis in Publication 23 is on data for radiation workers, and the new emphasis reflects the fact that radiation doses to the whole population are of increasing interest to ICRP and national bodies interested in radiation protection.

The purpose of this letter is to solicit information from users of ICRP Publication 23, including workers in the medical and biological sciences outside radiation protection who have used the document. In order that the revised document may be accurate and complete, it is important that we get advice from as many users as possible. The present task group would appreciate hearing about any errors, omissions of important information or shortcomings in Publication 23 or of relevant new data not included.

Please write to the task group chairman at the address below.

C.R. RICHMOND
(Chairman)

Task Group on Revision of
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Creationism

SIR — As our organization, the Association for the Protection of Evolution (APE), has been accused of misrepresentation and an ethical lapse by Professor E.H. Andrews in his response (*Nature* 312, 396; 1984) to our report of the Biblical Creation Society (BCS) open day (*Nature* 311, 703; 1984), we feel obliged to reply. First, if Professor Andrews claims to be a theist, why is he engaged on a project involving natural theological argument and the acquisition of empirical evidence from the natural world for the existence and putative activities of a God — a paradigmatically deistic position implicitly shared by all creationists whether they understand the meaning of the word or not? Second, Mr Malcolm Bowden (for it was he) has on several occasions castigated BCS for its dangerous backsliding tendencies towards the position of theistic evolutionism. Third, Andrews waxes

indignant at the suggestion that he has criticized US "scientific" creationism; we need only quote from his seminal article in the BCS "journal", *Biblical Creation* (vol.5, no.16) to show that he has been criticizing US creationism for some time. In this, the first "Biblical Creation Lecture", he writes "the American branch of creationist movement (*sic*) has therefore attempted to develop a scientific creationism that has no religious content". He goes on to say that such creationism involves "a number of very real dangers and problems", is "a-Christian" and "devoid of biblical content". His lecture (which we have on tape) merely amplified his previously published criticisms.

Chris Darnborough was introduced as doing research on genetic engineering and evinced an adequate knowledge of modern genetics, unlike all the other creationists we have so far encountered. APE made a theologically acceptable *in pectoris* decision to elevate Darnborough to the honorary position of BCS's top geneticist in default of any other candidates. The title of his lecture was, after all, "Genes created but EVOLVING" and he did have to apologize to his audience in advance in case they mistook the bulk of his lecture as putting the evolutionist view.

As for APE's "ethical lapse", in reporting Dr Gower, he did indeed state his belief that faults in the perfection of the "design" of the human olfactory organ are due to the effects of the fall of Adam and/or the work of the Devil. Whether he believes this enthusiastically or unenthusiastically is a matter of judgement. Our opinion was the latter. In any event, APE explicitly informed the organizers of the conference that it would be reporting their deliberations for the scientific press.

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SIR — The article "Louisiana law thrown out" (24 January, p.257), described the US court system's rejection of a law that would have required teachers to include "creation-science" along with "evolution-science". The law defined "creation-science" to be "the scientific evidence for creation, and inferences from those scientific evidences". This law required that students be given crucial data, even if they support a theory contrary to the beliefs of the teacher. Teachers who believe the creation account of origins would be required to include the evidence for evolution, just as the proponents of evolution would be required to discuss the evidence for creation. The rejection of this law en-

courages censorship of data about origins.

The inherent religious nature of *all* versions of origins must not be minimized. It is true that the creation account of origins is consistent with some "theistic religious views". The theory of evolution is consistent with the tenets of non-theistic religions as well as the tenets of the "main-line" and "liberal" churches. All views of origins support someone's religion: there is no religiously neutral theory of origins.

Students have a right to know that the lack of intermediate fossils discredits Darwin's theory of evolution. Students should also be informed that the modern belief in molecular evolution is held in spite of the fact that real observations require the rejection of spontaneous generation. The causes behind the shift in opinion away from creation in the 1850s should be reviewed and discussed with students. The citizens of Louisiana wanted to ensure that crucial data, such as the gaps in the fossils and the Law of Biogenesis, will be part of public school curricula.

The students must be able freely to form their opinions without the fear that critical data have been withheld. It is sad that one federal court and the American Civil Liberties Union seem to disagree.

RUSSELL T. ARNDTS

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Missing mass

SIR — I would like to make only a point of general scientific method in reply to your reviewer who found "glaring errors and omissions" in my book *The Hidden Universe* (*Nature* 24 January, p.329).

The book attempts to explain the subject of "missing mass" to the layman. Surely the significant point about this subject is the small but increasing body of observational evidence that has now brought sceptical astronomers round to believe in this phenomenon. The point is *not* the plethora of mythical particles, or other agencies, which can all too easily be advanced to fill up extragalactic space. Precisely because we know so little about the phenomenon, it is of course at present hard to test out the presence there of axions, heavenly chariots or discarded bandwagons. That being so, economy of effort alone directs our attention first to those candidates that are either known to exist, such as neutrinos, or at least lead to some testable predictions. Supersymmetry and GUT [Grand Unified Theory] particles may be appealing to some, while reminding others of epicycles. For the moment, GUTs have fluffed their first proton-decay test, and the fact that they contain 21 readjustable free parameters could be held to lessen rather than add to their appeal in the context of missing mass.

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Robots and images galore

Japan's massive science and technology fair, Tsukuba '85, opens this week in Tsukuba Science City. It is well worth a visit.

AT the end of the movie showing in Toshiba's pavilion at Japan's Expo '85, a young Japanese boy turns to a white-coated scientist and symbolically hands him a baseball. A robot with expressive eyes who has guided him around a research lab looks on, cooing electronically. The boy has decided to put aside his dreams of becoming a baseball star and become a scientist. The overwhelming message coming from the Japanese pavilions at the international exposition — on the theme "Dwellings and surroundings — science and technology for man at home" — is "Love science and technology".

The message comes in various forms:

"The affluent life of the twenty-first century begins amid the harmony of life and science", whisper the loudspeakers in Mitsubishi's "Wonderful world, beautiful people" pavilion as viewers ride cars on a trip beyond time and space.

"As management systems advance, accuracy and safety will increase, this will allow everyone in the community to enjoy life", says the commentary in Hitachi's rotating Interface Theatre.

"Technology can be fun", say the slogans in the Fujitsu pavilion.

And the epilogue in the government's history pavilion is "While each part acts independently, the whole functions with a beautiful order; just as the relation between the body and the cell so the relationship between society and scientific technology will grow". Alongside the message a sculpture of waving disks on tall, flexible poles demonstrates overall harmony in independent movement; surrounding video monitors show aerial views of *masu geemu* (mass games) of people on a playground — only when viewed from afar can the overall order in apparently chaotic movement be discerned; ever-changing flower-like patterns emerge. "This is the future harmony between man and technology", explains a guide clad in a silver uniform cut into geometric shapes.

The Japanese faith (officially, at least)

in the benefits that technology will bring seems untroubled by the doubts entertained in other nations; indeed, perhaps it is just this naive faith the Expo sells that will produce the miracles it predicts.

At any rate the amount of organization and effort that has gone into the Expo is truly spectacular, as is the resultant 250-acre site covered with futuristic pavilions from the Japanese government, 28 Japanese corporations, 47 foreign countries and 37 international organizations. Spectacular too is the number of people expected to attend. Recent surveys suggest that the original guess of 20 million visitors during the six months of the Expo is on the low side, more like 26 million people are likely to attend — more than one-fifth of the entire Japanese nation. What better way to accustom people to the high-technology future that lies ahead? That is, if all 26 million people can get there — huge traffic jams at the preview suggested that it is not going to be easy.

The Expo has not been cheap to set up. Some 210,000 million yen (\$840 million) has been spent, with one-third of it coming from private industry and the rest from the government. The official theme of the Expo, on "Dwellings and surroundings", seems not to have been taken that seriously. At the very beginning, it had been intended to hold an Expo simply on the theme of science and technology, but it had been pointed out (by the controlling body for international expositions in Paris) that this would virtually bar all but the advanced nations from participation. Thus the adoption of a more specific theme; it was thought (by a somewhat dubious bit of logic) that the less advanced nations could display technology appropriate to their particular "dwellings and surroundings" and the emphasis on high technology lessened. Indeed, that and very generous financial terms offered to the developing countries by the Expo organizers has ensured greater Third World participation than at any previous Expo. But for the Japanese contributors to the Expo — the two government pavilions and the 28 corporate pavilions that dominate the Expo and take up around 70 per cent of its space — it is still fundamentally an exposition of science and technology pure and simple. A walk around two-thirds of the pavilions revealed a model of only one dwelling (a solar house), no contribution from construction companies, and rather little in the way of new items for the home; the emphasis is rather on the boundless (and often rather

abstract) advantages of high technology presented with all the razzmatazz of a fair-ground.

In building the pavilions, and the corporations have lashed out around 2,000 million yen (\$8 million) on each of their own, Japan's top architects have been put to work. Or rather the top well-established architects have. Despite looking to the future, Japan's young architects have not had a look in; it is Kurokawa Kisho (who designed the Japan exhibition held at the Royal Academy in London a couple of

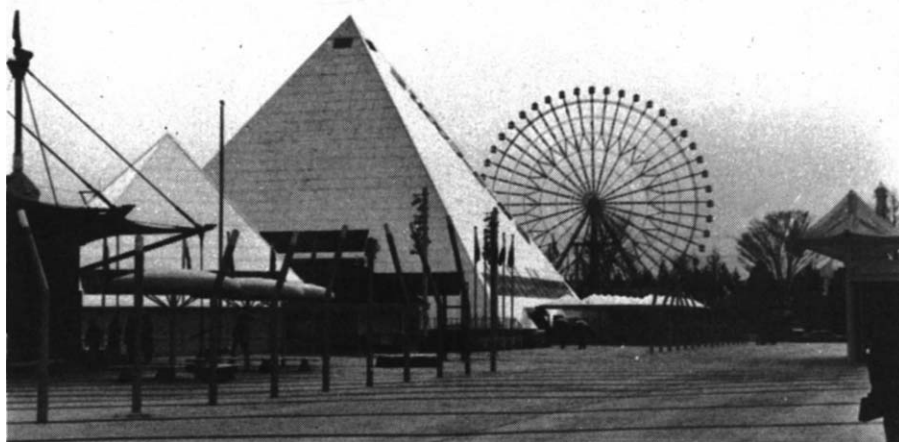


The Tsukuba Expo abounds in the world's biggest smallest and fastest. This is the Sony Jumbotron, the world's largest television. Some 25 metres high and 40 metres wide, beside projecting futuristic images it will be used for a world news service.

years ago) and Maki Fumihiko who lead the pack. The result has been a plethora of pythagorean solids: cones, spheres and pyramids galore dot the site. The IBM (Japan) building manages cleverly to combine a cone, a sphere and a square; the Toshiba building's ground plan is of a square, semicircle and triangle. Clearly the days when geodesic domes were inextricably linked to visions of the future are over; the only big domes are the two of the Midori-kan's biotechnology pavilion, linked together to represent cell-fusion technology. That is not to say that curves have been entirely rejected; the government Expo Plaza building looks like a giant white butterfly come to rest — a pair of outstretched scalloped wings roof an open space for the performance of festival shows. The US pavilion, by Maki, evokes a graceful series of billowing sails and the Electric Power pavilion consists of dozens of white tent-like structures with sharply-pointed peaks pulled out by wires running to the top of a tall, slim tower.

Tsukuba Expo '85

THIS feature is by Nature's Tokyo Correspondent, Alun Anderson, except where indicated. Japanese names are written with the surname first. The International Exposition, Tsukuba, is to run from 17 March to 16 September. The Expo site can be reached from central Tokyo in 2 hours.



Behind UCC coffee company's pyramid can be seen the world's largest ferris wheel with a height of 85 metres. Each gondola can carry eight people. There are worries that it may be struck by lightning, as the plain surrounding Tsukuba is notorious for mid-summer thunderstorms. The organizers assure doubters that it is absolutely safe and that the odd thunderbolt will only add to the excitement.

Traditional Japanese touches are provided too, again by Kurokawa. A long line of simple buildings with walls of cream ceramic squares set in black steel frames recall the sliding screens of traditional Japanese houses. A few pavilions have aimed at the downright exotic: Shueisha has a massive mock stone building covered in primitive carvings and exhibits a mummy from the British Museum for its theme of "Historical proof of Man"; and Dai's "The home of the poet" building is a low, stepped pyramid with its entrance hall underground.

Like expositions elsewhere, almost everything on the site is to be torn down when it is over (the area will become an industrial park), so the building of fantastic and ephemeral forms has been encouraged. It is impossible not to be impressed by a walk around the site, not only by the originality of the buildings themselves but by the sense of space with which they have been laid out.

Strangely, although the government has repeatedly said the whole exposition will present a "vision of the future", in actual fact no coherent picture of what future life may be like emerges. There are some individual exhibits — like that of NTT's Information Network System — that deal with bits and pieces of the future. But mostly on display are images — on giant screens, in 3-D, on high-definition televisions — and robots.

In the Fuyo robot theatre there are fifty self-propelled robots of twenty different kinds (but mostly of a very cute kind that only children will be able to tolerate), a talking robot roams the main open space (and switched into English while talking to this correspondent and followed him shouting "I want to go to England"), a robot plays the organ and reads music in the theme pavilion, Matsushita's robot draws portraits after a ten-second look at a face, Hitachi's assembly robots get out of the factory and spend their time sculpting ice blocks, Toshiba's robots spin traditional

tops and can send them along tightropes to one another, Fujitsu has the world's largest humanoid robot. More than science and technology itself, maybe it is robots that you must learn to love!

But why, in Japan of all places, has it been found necessary to go to all this trouble to inculcate a love of science and technology in the citizenry? Clearly, both industry and government have already decided that Japan's chief hope for continuing rapid growth and prosperity lies with high technology — innumerable government reports and exhortations have already driven the point home and the key products for the new era are on display at the Expo — intelligent robots equipped with sensors, electronic imaging equipment, machine translation systems, high-definition television.

But strangely, when seen against Japan's volume of high-technology exports, the people of Japan have perhaps had less contact with the new technology than elsewhere, and science and technology are still very much seen as the domains of "experts". Take some of Tokyo's most highly automated big offices for example; they may boast that they lead the "OA" revolution in Japan but the fact is that workers spend rather little time at electronic keyboards and rather a lot of time in old-fashioned face-to-face group discussions. Often companies are not themselves using on a large scale the technology they are selling abroad. Again, small computers are very much more widely diffused in schools and homes in the United Kingdom than they are in Japan. An exhibit in the British pavilion claims that "Young girls and boys in Britain are now learning the language of new technology as naturally as their mother tongue"; given the number of small computers available in Britain it may well be true. The same could not be said of Japan, even though Britain's computers are doubtless full of Japanese microchips.

Also the pace of Japan's industrialization has left some groups out of touch. The

rapid post-war shift of population to the cities has left behind a population in the countryside with a very different level of contact with high-tech; older people, too, feel more threatened by technology and will have had a much clearer experience of its ill-effects. Those who lived through the 1950s and 1960s, when nothing was allowed to stand in the way of high growth, will have clearer memories of Japan as the pollution capital of the world: Minimata disease, photochemical smog, arsenic accidentally introduced into milk, Itai-Itai disease....

In one sense, then, the Expo aims to strengthen a national consensus that the only way to move ahead is with high technology, to prove that science is not something "dry and stiff" but is something to enable people "to live in happiness". The Expo too is a festival celebrating just how much Japan has achieved already and how much technology is now part of daily life.

Apart from a little revision of history in one government pavilion, there is no evidence of nationalism or of a desire to boast of Japan's very remarkable achievements. But Japanese visitors to the Expo must feel an immense sense of pride in the wonders that are on display there. Perhaps it will bolster their sense of optimism for the future too.

The Expo also serves several subsidiary purposes, the principal one being to put Tsukuba Science City, on the fringe of which Expo stands, on the map. For all its



Nature's Tokyo correspondent as seen by Fuyo's robot.

46 laboratories and institutes, its two universities and its 34,000 scientists, and the million million yen that has been spent on it, it still has not captured the imagination of Japan's citizens (see *Nature* 305, 378; 1983). Perhaps the Expo will make it seem a brighter, more exciting place and help attract the population it is still lacking.

The style in which propaganda for the twenty-first century has been put over is very typically Japanese, even though the Japanese themselves may not be aware of

it. Two separate but entwined influences can be seen at work. First, there are the two trends in Japanese art (and life): towards extreme understatement, the ultra-refined, and the use of absence and emptiness to suggest presence; and towards the noisy, the colourful, the boisterous and the vulgar.

The first world is that of traditional Japanese gardens where a few rocks and some carefully raked gravel suggest a universe, of the exact placing of a few flowers in *ikebana* (flower arrangement), of the slow and subtle gestures of the masked actor in a Noh play, and of careful understatement in speech. This side of Japanese aesthetics is the better known in the West. But the other extreme also exists of wild and drunken festivals, of colourful Kabuki plays, and of grotesque and garish Edo-era paintings of actors and prostitutes and demons. In origin, the two cultures belong respectively to the samurai that ruled Japan throughout its feudal period, and to the common people, particularly those of Osaka and Edo — what is now downtown Tokyo.

Much of Japan's commercial television seems to spring from the latter source: there always seem to be two or three people shouting at once, lights are flashing, and girls are shrieking in an endless party from which everyone with a trace of introversion has been excluded. But a walk around Tokyo reveals that the opposite trend has by no means left Japan's everyday culture. Take a famous fashion chain store: passing by one of their shops a foreigner could be forgiven for thinking it had gone out of business. All that can be seen is a large marble slab with a single black garment on it, the racks of clothes for sale are kept out of sight so that they may not intrude on the elegance of the bare interior.

Or take the following television commercial. A view of a wall full of windows fills the screen; as the camera pulls gradually back it is revealed to be the side of a tall American skyscraper; a single long strip of paper falls from the sky, twisting and turning as it drifts down through the centre of the screen — and is then replaced by the name of a brand of mayonnaise, without any apparent connection to the images that have gone before.

What this commercial illustrates is not

The theme: science cool and wondrous

THE spirit of refined taste is one facet of Japanese culture that is seen at its best in the government's Theme pavilion. There is no clutter, nothing obtrudes and there is often a remarkable sense of peace; even the couple of monitors showing videofilms do not sit clumsily by displays, they recline in elegant glass cases as though exhibition pieces in their own right.

The first hall, Sun and Water, is high, light and airy and contains what has become through intensive media exposure the star of the Expo: a giant tomato plant. Grown from just one seed, the plant will demonstrate the potential of hydroponic farming techniques; if all goes well it will reach ten metres in height and produce 12,000 tomatoes by the end of the Expo. So far it is growing on schedule, and managed 30 tomatoes for the press preview two weeks before opening day.

The second hall, Man, is more surreal. In semi-darkness on a central octagonal platform, a silver, metal man-like robot plays the organ (a little soullessly) from sheet music read by a single television eye set in the centre of its head. To one side, wall panels show an ever-changing pattern of nude human bodies — the female conveying "grace, beauty and warmth" and the male "strength and vitality"; to the other side a quadruped robot looking like a gigantic black ant crawls slowly up a staircase, a frill of sensors around its feet feeling for the next step; behind paces a

strangely truncated bipedal robot — just a pair of walking legs ending in a squat metal box.

The last hall, Outer Space, is dark and cathedral; images are projected onto a screen from an Earth satellite while a golden model of it rotates silently alongside. On a moving walkway, one sails slowly across a vast elongated screen filled with images of molecular structures, planets, suns and nebulae that turn into the radial arms of colossal space stations. The journey ends in a corridor of sloping mirrors and bright red fine scaffolding that gives one the impression of having landed inside an infinite cubic lattice.

Along the way there are quite a few objects: the Shinkai 2000 deep-sea vessel hangs in the "Sun and Water" hall, and the silver multiple compound eyes of the solar ray concentration and transmission system stand at its centre. An NMR scanner, a laser scalpel and an organic holography display stand quietly in the "Man" hall. The second stage of the HI rocket has a corner in the "Space" hall. A separate open area — a future town plaza — in elegant greys has a model solar house and a city of the future.

But the real emphasis is not so much on things or on how they work, or on presenting a glimpse of the future; it is more to create an almost religious state of awe. Whatever science and technology is, it is something strange and wondrous. □

just a partiality for minimalism, but a second trend also seen at work in the Expo, perhaps best caricatured as a "disinterest in facts". However many commercials you see on Japanese television, you would be hard put to find one that gives you any facts about the product — or even a good reason why you should buy it. As an advertising agent who boosted Sony's Walkman to success put it, "we stress the emotional benefits of the product, not the physical properties". Everything aims at the evocation of an ambience, a mood to be associated with the product — not at conveying information about the product. Indeed, Western society is often described as too oriented to facts, what the Japanese call "dry". Instead, they are (or like to

think of themselves as) "wet", as being primarily concerned with their emotional reaction to things.

At individual Japanese pavilions in the Expo, the tendency towards extremes of understatement and over-statement can be clearly seen. So too can the tendency to rely on emotion rather than logic. For Westerners the chief criticism of the Expo would be that it dazzled rather than informed. One suspects that many Japanese would not really care. A Japanese corporate participant apparently shrugged off comment of this kind with "However educational and profound the theme may be, it's a waste of time and money if no visitors come". So the emphasis is on spectacle, fun for all the family, and slogans. There is another important consideration; the crowds are expected to be so huge that if anyone stops to press a button or think too long it might bring the whole Expo to a halt!

Nevertheless, there are many thought-provoking exhibits at the Expo and some of the best (for this reviewer) are described in the pages that follow. Pavilions of foreign countries, too, present their own individual styles — many of them the "high information" style that is more common in the West. It will be interesting to see how the Japanese react to them, and how the million or so foreign visitors expected to come react to Japanese exhibits. All in all, worth coming a long way to see. □



Japan Airlines' experimental High-Speed Surface Transport, on view at Expo, uses magnetic levitation for lift and a linear induction motor for propulsion at up to 300 kilometres per hour.

History pavilion

A little judicious rewriting

GIVEN the overall aim of the Expo to create a new feeling of warmth towards science and technology in the hearts of Japanese citizens, it is not surprising that the view of the past should require a little revision. In the process, some old and misleading myths about Japan have been thrown out.

What the first half of the government's "History" pavilion does is to chart factors responsible for the first Japanese miracle: the very rapid modernization of the country after it opened its doors to the West and abolished the Shogunate in 1868. Indeed, progress was so fast that within the space of a few decades Japan went from an isolated feudal country to a major power that was capable of defeating the might of the Imperial Russian army and navy.

Understanding of this leap forward is particularly important for developing countries, especially those in South-East Asia, who frequently try to take Japan as a model for their own development. The exhibition makes it clear that success did not spring from unprepared ground and was not due to some inexplicable spirit of the Japanese. First and foremost, even before direct contact with the West, Japan had a population that was highly educated and literate. Information panels (unfortunately written only in Japanese and thus inaccessible to foreign visitors) show how there was a major boom in small *terakoya*

schools which gave basic education in reading, writing and the abacus in the early nineteenth century; by the middle of the same century it was almost certainly true (as it still is) that the Japanese were the most highly educated people on Earth.

It was to this relatively enlightened population that the best of Western technology was brought in to be imitated and improved upon. A series of intelligent decisions were then made to allow technology to disseminate quickly; some of the very first university engineering departments in the world were opened, systematic methods of measurement were introduced, a patenting system set up, and industrial standardization was adopted early on.

The defeat in the Second World War destroyed the material fruits of this effort — then came the second economic miracle and Japan's current technological successes. Explanations for these are much more abstract and fall back into clichéd explanations about the "special characteristics" of the Japanese people.

Beautifully arranged exhibits of traditional lacquerware alongside the Sony Walkman show the Japanese sensitivity for "lightness"; a stylized Zen garden illustrates the Japanese desire to "control time and space"; a *karakuri* doll — a kind of sophisticated mechanical doll worked by levers which was first produced in the seventeenth century — is shown with a cap-

A touch of class from the British

LACONIC understatement, either a virtue or a character trait, marks the British pavilion. The designers have set out to ensure that visitors will not be impeded in their hunt for another stamp on their Expo passports by buttons crying out to be pressed. Instead, there is a cunning array of video screens hidden in structures as different as breaking waves and seashore rocks. Backward-looking (or historical) information has been avoided as if the plague. And to avoid carrying coals to Newcastle, the planners have made much less of the British government's domestic ambitions for information technology than might have been expected. But Sir Clive Sinclair has been persuaded (apparently reluctantly) to put the latest version of his Spectrum computer on display, while the UK programme for encouraging the use of computers in schools is heartily endorsed.

The first objective of the exhibit seems to be to let people know what Britain looks like, both to the eyes of visitors (accomplished by means of life-size cut-out models as in those of pop-up children's books) and by Landsat images collected from Earth orbit. If necessary, the exhibit will also demonstrate to visitors exactly where Britain is, apparently a matter on which

many Japanese are uncertain.

Environmental protection, typified by the return of salmon to the Thames, looms large, as do some of the recent developments in medicine (NMR scanning, for example) and pharmaceutical research (H_2 antagonists for treating gastric ulcers, for example). The IRAS satellite is represented by a model that could well have been larger, but the infrared image of the Milky Way is as arresting as anybody could wish.

On balance, the impact of this modest display on visitors is likely to be pleasing and reassuring. Even before the video displays had been switched on, and the lights darkened, the sense of unpretentious orderliness was clearly apparent. Some may think that the decision not to look backwards notwithstanding, more might have been made of British contributions in basic research — in molecular biology, for example. But what, in any case, will stick in people's minds is the largest single object on show, a sleek Jaguar coupe in royal blue. Apparently the taste for these machines is catching on even in Japan, with the result that one is to be seen most days on the neighbouring parking-lot, the property of the manager of a nearby pavilion.

John Maddox

Pacific rivals

AUSTRALIA and Korea inhabit spacious pavilions, as befits fellow-Pacific states. But there the similarity ends. Australia plainly wants Japan to know where it came from. Korea's message seems to be that Japan should watch out for a rival to the West, and in the meantime spend its vacations in Korea.

Australia's history comes across in sepia-tinted blow-ups, with as full an account as anybody could ask for of the precedence accorded to the aboriginal population. Heroic eighteenth and nineteenth century settlers create a melting-pot to which Asian populations have since been added. Now there is a modest boast about Australia in astronomy, and about the keel that won the America's Cup from the United States. A dashing video display says perhaps only half of what might be said about the splendour of Australia as a place. Korea, by contrast, makes itself seem a little like Japan but more exotic (which is fair). Its high-tech products are relatively modest — cars, telecommunications equipment and so on. But the similarity is in itself enough to suggest the threat of competition from around the corner.

John Maddox

tion which links past interest in these dolls to the modern achievements in robotics (the "robot spirit"?). Japan is one of the few countries to endlessly dissect its "national spirit" to define its unique characteristics — the doll exhibit is as silly as one would be that put Victorian English miniatures alongside microchips (the technology was invented in the West, not Japan) to prove a British "love of the small".

Curious too is an exhibition of traditional technologies. A staggering set of six multiple projection screens with an overpowering sound system dominates a great dimly-lit hall at the back of which giant water wheels slowly turn. On the screen, flashing images of traditional sword-making, and from the loudspeakers a mighty cry as the red-hot sword is plunged into clear water. The message is a very fair one; that in the past Japan had independently developed sophisticated technologies. But the exhibit captions go beyond this and seem to suggest that there is a native Japanese science and technology, a scientific tradition that is uniquely its own; as though there were a difference between "science" and "Japanese science". Lying behind this there is perhaps some feeling that "science" is still in some sense an import and that proving there is a Japanese science will somehow make it more acceptable or lovable.

Having set out to destroy some myths about one miracle, it is a pity to see the exhibition introducing new myths along these lines. How much better the IBM (Japan) pavilion's attitude to history; Japanese scientists, as fine as any other nation's, take their equal place alongside foreigners in one international science. □

Corporate pavilions

Electronic extravaganza

GOING over the top to prove that technology can be a barrel of fun are the big Japanese electronics corporations — Toshiba, Sumitomo, Matsushita, Hitachi, Mitsubishi, Mitsui and the Electric Power corporations. And for children and their mums and dads on a day trip to the Expo the whole experience probably will be great fun and a lot closer to a day at Disneyland than at a science museum.

The first three corporations offer films as their main attraction, the last three rides — a kind of high-tech ghost train — and Hitachi in the middle combined the two with a rotating movie theatre. Among the rides the "Electrogulliver" trip offered by the Electric Power corporations is by far the most unnerving.

The ride begins with thirty seconds of complete and total darkness, after which the chair makes a sudden lurching descent and its occupant is hit by a fine spray of cold water. This turns out to be preparation for a quick steam in a very hot volcanic magma chamber. After that the traveller goes through a forest inhabited by twitching purple cockroaches and into the heart of the Sun to participate in a nuclear fusion reaction — an environment available in 3-D and computer simulation in pavilions elsewhere, so the visitor to Tsukuba soon becomes perfectly at ease with a few protons flying excitedly about.

Elsewhere there are rides into space and past black holes, and in Mitsui's water theatre the adventures of a boy and robot, incorporating a film projected onto a giant circular waterfall surrounding the ride.

Indeed, exhibitors opting for film are nowhere content with mere conventional projection systems. 3-D is everywhere. Sumitomo boasts Japan's first purpose-built giant 3-D screen and a moving tale of a little girl and her sheepdog "Bozo", and Hitachi the world's first 3-D colour computer graphics and a curiously old-fashioned view of the future, with robots vacuum-cleaning and scolding children for not doing their homework in a story much like that of comic strips of 20 years ago. Toshiba has a super-reality theatre using film taken and projected at three times the normal speed for the ultimate image. Special effects are provided by the maker of those for "2001" and "Close Encounters of the Third Kind" and contain the best robot ("Pal") in the Expo.

Some pavilions also have some technology on display. Toshiba's robots are particularly impressive. One inspection robot and five one-armed assembly robots deftly spin traditional Japanese tops and make them walk a tightrope. Alongside is state-of-the-art medical imaging equipment — magnetic resonance, tomographical X-ray and ultra-sound — and an astonishing pattern recognition machine that can sort out foreign banknotes at the rate of 1,500 a minute. To make the machine more lovable for the Expo, it does not actually touch any money but sorts out five kinds of cards depicting scenes from fairy tales. Unfortunately the machine still looks for all the world like a giant metal-and-glass sarco-phagus — a few pink ribbons might have improved it.

Most pavilions rely simply on spectacle. You certainly will not learn any science from them (or see much real technology)



Film projection onto falling water is Mitsui's main attraction.

and Westerners are in danger of emerging with a fuse blown somewhere. But Japanese children have been reared on their local television and are made of sterner stuff. They will be queuing up for more until the Expo ends. □

NEC pavilion

A place for galactic heroes

FUN and games with the future, the insistent theme of the Japanese pavilions, will be eagerly sampled at the Nippon Electric Company (NEC) pavilion, most easily recognized by the 32-metre parabolic antenna which towers above it. There was some embarrassment, on the day it snowed heavily at Tsukuba the week before opening day, that workmen had to keep the crowds away from the lumps of slush falling from the rim until the dish could be pointed to the zenith.

NEC has built a theatre for playing interactive computer games. Visitors are given a piece of plastic, not called a credit card but called a boarding pass, that will activate a small computer terminal in front of every seat. A film whose script begins with Neil Armstrong's observation on landing on the Moon puts the audience into a space ship whose navigators repeatedly run into swarms of meteorites and, now and again, a black hole. At the approach of each potential catastrophe, everybody's seat vibrates. Those who make the wrong choices of a strategy for escaping find that their seats tilt back alarmingly. Half-way through the show, there is an exercise at destroying meteorites with a ray-gun, when each terminal records its user's score.

Crude though it may be, this jazzy show cannot but be a hit. NEC has probably underestimated the queues there will be, with merely 30 square metres set aside outside for standing in line. But those who will be waiting under cover will be treated to a 5-minute quarrel between three television sets whose screens are not merely filled with cleverly drawn animated faces, but which can nod and shake so tellingly that even those who cannot understand the Japanese will think them alive.

NEC's motto for the present is "C & C", which means computers and communications. The company has set out to simulate something of what it thinks the next century will be like in what it calls its Techno-Plaza. There is a vehicle with a computerized guidance system that locates it on a moving map display, and which tells the driver when and which way to turn at intersections. Just as well, visitors will conclude, for the driver will otherwise be too busy using the tricks with which the vehicle is equipped for sending documents back to head office and retrieving data from the databanks. NEC apparently has no immediate plans to put its car on the market.

Two of the exhibits are more telling: a group of three six-jointed robots, or simulations of human arms, which are wired and programmed in concert to carry out co-operative tasks, and the prototype of a telephone interpreter that will convert spoken Japanese into spoken English. NEC has for some time been putting a large part of its research and development into artificial intelligence. If the rough edges of its interpreting telephone can be smoothed out, it may not have to wait until 2000 to have something to sell.

John Maddox



Four million pairs of 3-D glasses will be given away during the Expo.

Fujitsu pavilion

Toughest robot in town

What mankind can dream, technology can achieve ... Robots act for us, machine translation systems think for us ... computer imaging technology simulates realities beyond our realm of perception.

ALTHOUGH Fujitsu falls in with most other corporations in its glorification of technology, it does so with a little more style; some interesting exhibits are on display and its 3-D film is streets ahead of anyone else's.

Dominating the exhibition is the world's largest humanoid robot, Fanuc Man; 5 metres tall, weighing 25 tons, and kept in a special cage which makes it look as though it is eager to break out and start climbing skyscrapers. But Fanuc Man is,



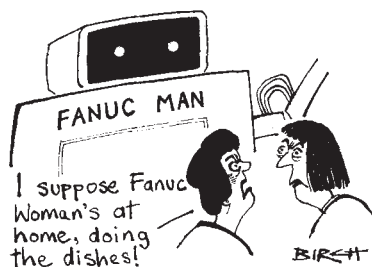
symbolically, both gentle and strong. For the amusement of visitors it alternates between the dexterous task of assembling a one-thirtieth scale model of itself, aided by television cameras set into its hands, and pressing a 200-kg barbell (not by sheer brute force but by careful load balancing). Fanuc Man and his like are destined to assist one-armed robots who spray paint and spot-weld cars with the assembly of heavy components — doors, seats and so on.

Japan's lead in robot production is pretty much unquestioned. But software has been considered a different story, with Japanese thought lagging behind their US and European counterparts in creative ability. Fujitsu makes that point but claims that the software used at the Expo shows just how quickly things are changing. Fujitsu believes its machine translation system to be the world's most advanced and it is set to work at the Expo translating Japanese sentences — written by children at a booth on their way into the pavilion — into English, French and German for projection on a screen elsewhere in the building. A Fujitsu engineer was happy to admit, though, that even a short sentence takes 15

minutes of mainframe computer time to translate. Just why is not hard to see. Japanese is written without breaks between words and the first step must be to segment the sentence into words through a dictionary and information about the adjacency relationships of words; only then can a context-free grammar be put into use to parse the sentence and identify grammatical elements. Next comes the really difficult bit: the computer has to have some sort of commonsense world model to figure out which particular meaning of each word is intended — it has to "understand" the sentence — before a translation can be attempted. Fujitsu uses the semantic network approach; essentially a hierarchical grouping of words with linked meanings (a bit like the organization of a thesaurus rather than a dictionary), as a part of a knowledge base. It is after this stage that words from a different language with corresponding meaning can be selected.

Clearly the problems are very complex and general purpose machine translation with enough knowledge to handle the rambling sentences written by real people is a long way off. But in restricted fields where ambiguity has been deliberately eliminated, progress is expected to be more rapid. Within five years, Fujitsu expects computers to handle almost all translation of technical manuals; technical translators may be the first highly skilled white-collar workers to be made redundant by computers.

The other software advance of which Fujitsu is keen to boast is that used in generating the company's new 3-D film — the world's first 3-D computer graphics film for projection on a dome. The film depicts a journey inside ice crystals, through the planets, and into the Sun to see the mechanism of nuclear fusion, and ends with a sensational simulation of a DNA molecule being wound and rewound and re-



packed until it forms a chromosome. Days of computing time on one of the world's largest and fastest supercomputers were needed to generate this sequence; the intention was not to produce a cartoon but to produce exact simulations based on the most recent molecular models. Many a 3-D film at the Expo promises a trip inside a "different dimension" — Fujitsu's is the only one to achieve it. □

IBM (Japan) pavilion

A touch of wisdom

AMONG the domestic pavilions, IBM (Japan) would have to take the prizes for exhibits that are both fun and teach one something about science. Responsible for the overall organization was Nobel-prize winner Leona Esaki, who now works for IBM, and a team of top Japanese scientists—the difference shows. What IBM wanted to get over was something of the spirit of scientific discovery and of the way each scientist builds on what has gone before. The latter is attempted through a picture and text wall display of key periods and individuals in the history of science. The earlier periods — the Ancient Greeks beginning with Pythagoras, passing through the contribution of men like Avicenna from the Arab world to the Middle Ages and the revolution brought about by Brahe, Kepler, Galileo and Newton — were pretty much what might be expected. From then on a newer view of the history of science emerges.

First, there is the transition in the early eighteenth century when "the religious motivation to demonstrate God's will through the understanding of Nature's laws gradually gave way to the study of Nature for its own sake, or... for material benefits" — the age of Watt, Franklin and Cavendish. Then follows the Age of Specialization, that of Darwin, Maxwell and Edison, when scientists founded professional societies and "sought recognition through short papers on specific topics rather than lengthy treatises attempting to explain all natural phenomena as elements



The IBM pavilion takes its form from paintings of the nineteenth-century Zen priest Sengai — the circle, triangle and square respectively symbolize the Universe, human intellect and Earth.

of a single system". This was the period when *Nature* (the journal) was born — not that all its papers were that short.

In our own century came the Age of Technology, when immediate industrial applications came to be expected for research results and "military research in particular flourished" — a remark that would be commonplace if it were not the only reference to the military uses of science spotted in the whole Expo. And now we are in the Age of Big Science.

Along the way, Japanese scientists who had made a contribution to the world's science are introduced quite naturally, in contrast to the government's History Pavilion, which attempts to establish that there is a "Japanese science". The four Japanese Nobel-prize winners, Yukawa, Tomonaga, Fukui and Esaki himself, are of course mentioned but so are others who are not well known in the West but should be: among them Shizuki Tadao (1760-1806) who introduced newtonian mechanics to Japan, Kitasato Shibasaburo (1853-1931) who discovered the plague bacillus, and

Nagaoka Hantaro (1865-1930) who created a Saturn-like model of the atom.

To teach you something of what science is about there is a whole series of interactive displays of the type familiar in Western museums, with plenty of buttons to press. In a model of the atom, for example, it is possible to excite an electron into higher orbitals and see it emit a flash of light as it later drops back; on a giant globe one can manoeuvre continents around to follow different stages of the Earth's evolution. Scientific games are also available in plenty — played on a whole roomful of IBM personal computers.

The supposed highlight of the exhibition is a theatre in which 11 synchronized projectors show a film simultaneously on a domed roof and a giant sphere set in the middle of the hall, while spectators are carried around in a circular walkway. The film is designed to teach the relationships and dependencies that exist between man and science and not "simply to dazzle the visitor". All very proper and almost a rebuke to some pavilions nearby. □

NTT pavilion

Electronic future on display

JAPAN'S soon-to-be-privatized telecommunications monopoly, NTT, has seized the opportunity provided by the Expo to try and show that "INS will make life more exciting".

INS (Information Network System) is what NTT believes will be the telecommunications system of the future: optic fibre cable and complete digitization of the transmission systems will usher in the electronic age of videophones, home shopping and banking, instant access to databanks and electronic mail. A model system is already on trial in the Mitaka suburb of Tokyo (see *Nature* 311, 289; 1984); the very newest gadgets were on display at the Expo.

One instrument not seen before is the scopephone; a telephone with a couple of centimetre-square black-and-white video screens in its plinth and a miniature camera, revealed only by a small opening, under that. The scopephone does not need the high-bandwidth cable that handicaps ordinary videophones or videoconferencing facilities; of course, the picture is not of such high quality, and if the subject moves about too much the picture temporarily blurs before settling down again. But the scopephone gives a perfectly adequate sense of seeing the person you are talking to — and it is cheap. Chances are this could be commercialized on a large scale very soon.

Also new is a miniature fax (facsimile transmitter) built into a public telephone that achieves the transmission of whatever is written on a small piece of paper just the size of a visiting card; it will make it possible for the Japanese to extend their habit of introducing themselves by exchanging namecards even to a telephone call. As most Japanese bow even when

speaking on the telephone (the habit is easy to acquire and hard to break) this invention will make the ritual introduction complete — clearly someone in NTT's marketing division had a brainwave.

On display too is a videoconference room, the sketchphone (that enables one to transmit doodles as one speaks) and the CAPTAIN teledata system. But the big problem with all the hardware is a familiar one — what can it be used for? NTT tackled this question by asking what would have happened if the hardware had been available a hundred years ago: a series of sketches performed by actors in small studios around the pavilion gives the result.

One performance comes right out of one of the most curious episodes in Japanese history soon after the Meiji restoration had opened Japan to the West. The setting is the Rokumeikan — a banqueting hall and ballroom specially built in 1883 for the Japanese elite to entertain foreigners to Western-style balls in formal Western clothes. The balls must have been very uncomfortable for the Japanese but their purpose was very serious — to convince the long-nosed red-faced barbarians who had pushed their way into the country that the Japanese were as civilized as anyone else. At issue were the "unequal treaties"; treaties forced on the Japanese that ensured that foreigners who committed crimes in Japan could only be tried (or more often acquitted) by their own consular courts. The treaties were a source of great shame to the Japanese. In the event, a naive display of elegant manners and correct dress did not convince the foreigners that the Japanese were equals and the treaties were not revoked — rapid industrialization

Brainwaves to touch

WHY should Kodansha, a major Japanese publisher, have taken an area of 1,800 square metres at Tsukuba to build what it calls a "brain house", a multi-video explanation of how the human brain works? Because that is how people use its products, of course.

The result is an extraordinarily ambitious exercise in popularization. The designers have tried to represent what little is known of the anatomy and neurophysiology of the human brain in a series of working models; press a button and see how input to the right eye distributes signals predominantly to the left cortex, but partly to the right hemisphere. Other sensory systems, and the role of the hypothalamus and the pituitary, are similarly described. Perhaps the definiteness of the labelling (and the life-size and thus too-small models) may give visitors the impression that phrenology is back in fashion.

This sober stuff is submerged in one of the jazziest video displays at Tsukuba. The whole of a darkened dome is used to represent the way in which the networks of neurones are used in different physiological functions, while a character called Goku, derived from Chinese fairy stories but animated for these purposes, serves as an entertaining unacademic expositor.

As popularization, the pavilion succeeds in the sense of showing a lot of what is known. It conveys a sense of what remains unknown less convincingly. But it is a courageous attempt to explain a large chunk of science without promising that a host of gadgets will ensue. John Maddox

and military might did the trick a little later.

So much is known to every Japanese schoolchild; what the sketch shows is poor Mrs Itoh, wife of the first Prime Minister Itoh Hirobumi, worrying over what to wear, and how to dance, and what food to cook to please the foreign devils at the Rokumeikan. Of course, INS provides all the answers — instant access to video databanks through the high-speed video response system gives her dancing lessons and cooking lessons and doubtless many other insights into all our nasty habits. Information is power! The point is well made: Tokyo is these days full of foreigners clutching books on how to do business with the Japanese (and even how to hold chopsticks) and doubtless wishing there were video databanks to make it all a little less painful.

But NTT's video presentations of the future uses of INS fall short of being really convincing. A forester worried about how many trees to cut calls up an Earth resource satellite, a fisherman with access to a meteorological satellite — will it be cost-effective? With INS's more exotic applications, Japan has for once moved out in front of other nations. Will Japan discover that being a trail-blazer can also mean going the wrong way? □

TDK pavilion

Animals getting taped

MAKING strange the ordinary abilities (or events) we normally take for granted is perhaps the first step to becoming a good scientist: one Nobel-prize winning ethologist used to advise that one should try looking around one from the standpoint of a "Martian that just arrived on Earth". The pavilion presented by TDK (the magnetic tape manufacturer) comes close to simulating that spirit. It attempts through a film to give some taste of the strange experience of perceiving the world as if through the visual, auditory and touch senses of an animal.

The idea is one that has often attracted animal documentary film makers but is notoriously difficult to carry off. TDK's attempt is at its most successful in depicting the underwater world of fish, where changes in the field of vision, depth of focus and the absence of long range are easy to simulate photographically. The world of the horse, where the visual field is extended to 320 degrees in the horizontal plane but greatly narrowed in the vertical plane, is also easy enough.

Things get much more difficult when smaller forms of life are considered, the forms of which D'Arcy Thompson wrote: "A water-beetle finds the surface of a pool a matter of life and death, a perilous entanglement or an indispensable support. In a third world, where the bacillus lives, gravitation is forgotten. We have come to the edge of a world in which we have no experience and all our preconceptions must be recast."

Alas, after attempts to see what a rain-



The TDK pavilion is a model of a zokoncho (Elephinsect bird) which combines an elephant's trunk, an insect's compound eye and the flying abilities of a bird.

storm would be like for a small insect — an occasional encounter with a very large object — things come seriously unstuck as the film turns to the compound eye; an extraordinary kaleidoscope of multiple images fills the screen while a voice tells one that "it is thought the brain may process the images into one" (of course, this may have been a clever in-joke for troubled insect neurophysiologists). Nor does the film find any new way round the intractable problem of modelling auditory and tactile senses, other than explaining how they are better developed in certain species.

But overall, the film just about works, and at the end it returns nicely to technology by stressing how, from the study of animals with senses superior to our own, we can learn how to build new sensors. Respect Nature and learn from it is the message, one that got lost in the multi-sensory stimulation of most pavilions. □



Tokyo residents kept awake by the next-door neighbours' stereos would certainly be grateful if Matsushita can speed development of their speakers. Sound from the large circular speakers set a couple of metres above the ground can only be heard when standing within the smaller circle seen on the floor — a 50-centimetre movement of the head and all the sound vanishes. Normal sound is of too long a wavelength to be sharply focused in the same way that light can. Only ultrasound can be easily focused — but of course humans cannot hear it. So what Matsushita have done is to use two focused beams of ultrasound of slightly different frequencies. At the point of focus they interact with one another; the result of the sum and difference of the two waves is the generation of audible sound at that point only.

Suntory pavilion

Today birds, tomorrow men

AT last a discordant note in the hymns being sung to the boundless joys of technology at the Japanese corporate pavilions! At the Suntory pavilion not only is it acknowledged that technology can cause plenty of problems, but also that some of those problems need dealing with fast.

Suntory's must be the only pavilion to state clearly that curses have also sprung from technology: "ever worsening environmental pollution, wanton consumption of the precious blessings of Nature, a loss of concern for the protection of living things, a lack of warm regard for one's neighbours".

The pavilion's theme is "Today birds, tomorrow men", the point being made is that birds are among the living things most sensitive to changes in the environment and many have been pushed to extinction — after them it is us. To drive the point home, the pavilion employs the world's largest cinema screen and a film, concentrating on efforts to save wild birds, which contains what must be some of the best shots ever taken of wild birds in flight.

The film centres on a family of Canada geese and, in the most striking sequences, it follows them in flight across ocean and



Suntory's pavilion was shaped by bending steel pipes, one by one, into catenary curves. The surfaces are meant to evoke the image of a hill and to symbolize Man's desire to regain the beauty of the natural environment of the past.

forest, partly in extreme close-up from just behind the leading bird as the flock flies in a V-shaped formation soaring across the oceans. How did the photographer manage it? A Suntory representative could say only that a special "silent glider" had been developed by a Japanese-Canadian team to allow the mid-air close-up.

To its credit, the pavilion is part of Suntory's Wild Birds Campaign, which has been running since 1973. Suntory is a large whisky manufacturer and has built bird sanctuaries at many of its plants. No doubt it is good for the corporate image, but it is good for the birds too. □

CERN collider seeks the Centaurs

Last Sunday the proton-antiproton collider at CERN generated the world's highest energy interactions yet, in a quest for some strange effects first seen in cosmic rays. The work should continue.

AT 1 a.m. last Sunday morning, jubilant experimenters at the European Organization for Nuclear Research (CERN) working on the UA5 detector on the proton-antiproton collider recorded their first events at 900 GeV in the centre-of-mass, the highest energy at which matter has been collided with matter (or anti-matter) under controlled conditions on Earth. The object over the next few weeks will be to discover if the collisions show signs of the very-high-energy "Centaurus" ("centaur-like") events first detected in a Japanese-Brazilian cosmic-ray experiment in the Andes¹ as long ago as 1972, events that appear to lack all production of photons. Centauros are unexplained and treated with scepticism by most experimental particle physicists, but if they exist — and those who have studied the data carefully say they do — they would presage a fundamental reconstruction of theory.

Hence CERN's brief gamble, which, following an idea by UA5 group leader John Rushbrooke of the Cavendish Laboratory, Cambridge, involves a complicated ramping of the bending magnet current and accelerator cavities so that the colliding beams can reach — momentarily — 450 GeV apiece. The energy can only be maintained for four seconds at a time, as the magnets would overheat at the currents required to sustain the bending of 450-GeV beams. So the beams are decelerated to 100 GeV, and then brought up again to 450 GeV a few seconds later when the magnets have cooled sufficiently. It is undoubtedly an extraordinary feat of accelerator engineering, which pushes the collider energy up nearly by half from its usual constant 630 GeV maximum in the centre of mass.

The experiment certainly is a gamble, however, and CERN is treating it as such, giving UA5 the tail-end of the electricity supply year, when by contract *Electricité de France* can cut supplies to CERN for a day at a time — and usually does. There are five such *jours critiques* left before Easter, when UA5 must shut down for good. CERN staff describe experiments at such times as "crazy runs", but Rushbrooke said on Monday that he hopes UA5 will still collect some 100,000 events.

This should be enough to see if the Centauro effect is real — provided it occurs at or below 900 GeV. UA5 has already searched at 630 GeV but found no sign of the events. In the cosmic-ray experiment on Mt Chatacaya in the Andes, however, a per-

cent or so of the highest energy events recorded were photon-less Centauro events. (These events appeared unusually different in the upper and lower halves of the Chatalcaya detector — hence the "centaur" designation.)

Nevertheless, all the five Chatalcaya Centauros appeared to have equivalent centre-of-mass collision energies of 1,500–2,000 GeV, and it is uncertain whether, even at its boosted 900-GeV energy, the CERN collider will reach the threshold at which the Centauro effect sets in. (It is presumably somewhere between 630 and 1,500 GeV, if the events exist at all.)

At this point CERN's competitor steps into the act — the Tevatron collider at the Fermi National Accelerator Laboratory (Fermilab) near Chicago. Already this July and August, the superconducting Tevatron will take its first trial proton and antiproton beams, and the detector designed by Roy Schwitters of Harvard University and his collaborators will begin to take data at centre-of-mass energies that should run from 800 to 1,600 GeV. "It's a shakedown cruise" and "a very ambitious goal", said Schwitters last week, to get data on the Tevatron collider so early (the first serious data were not expected before spring 1986) and "we'll be delirious with joy if we see any collisions", but the chance is there that if CERN sees no Centauros now, Fermilab may see them this summer at its higher energies, or at least by the end of next year.

UA5 is worth a gamble, however — and not only to keep up the prestige of CERN (though prestige itself is not a negligible factor, as the vitality of a laboratory depends on it). UA5 is a unique detector, containing the world's largest "streamer chamber". This visualizes tracks as streams of sparks, rather as bubble chambers visualize them as rows of bubbles, and can resolve more detail in multitrack events than many other detectors, such as those under construction at Fermilab.

If, as the cosmic-ray experiments suggest, some strange global change is going on in the track composition and multiplicities at very high energies, UA5 may be in the best position to detect it. CERN might even be well advised, if the present run reveals no Centauros, to try boosting the energy yet further in the next "crazy run" period in March 1986, by improving the cooling of the bending magnets (though that could be expensive).

Fermilab, also, might do well to introduce steamer chambers to its complement of Tevatron collider equipment.

But this leaves out the question of the cosmic-ray observations themselves, and whether more can be done in that area. Undoubtedly physics smacks of the 1940s rather than the 1980s, as the events are rare and uncontrolled — as opposed to the billions of events that can be produced with tuned, intense accelerator beams. But they can be of almost unlimited energy, and still thus throw up new physics, as the Centauro events themselves indicate.

One member of the UA5 collaboration, Narendra Yamdagni of the University of Stockholm, has spent five years working with the Japanese physicists who contributed to the Centauro study, and is impressed with the evidence. This shows up not only Centauros, but also other ultra-high-energy phenomena such as "binocular" events, where the cosmic-ray collision far up in the atmosphere appears to produce two (or more) clear jets of products, and, more recently, so-called "chiron" events in which there appears to be a microscopic bunching of tracks within each jet. A Moscow collaboration working on Mt Pamir has also seen a Centauro event "even more spectacular" than the ones seen on Chatalcaya, says Yamdagni.

Yamdagni has now left cosmic-ray work to experiment on UA5 and the large electron collider (LEP) at CERN, but he strongly recommends that cosmic-ray research should be amplified, perhaps by flying 1-ton cosmic-ray experiments in routine passenger-carrying jumbo jets. "Their payload is 50–70 tons" says Yamdagni, "so it should be possible to accommodate a ton".

Cosmic-ray intensities in the stratosphere are 40 times those obtainable deeper in the atmosphere on Mt Chatalcaya, Yamdagni points out. Moreover, within a one-ton experiment, equipment (such as proportional chambers) could be included to make results less ambiguous than at present. It would also make them more acceptable to sceptical particle physicists. Japan Airlines are already flying, free, 20-cm-cube detectors designed by the Waseda University group that helped find the Centauros. Perhaps they should be 125-fold more generous, and extend the dimensions to a metre. **Robert Walgate**

1. Lattes, G.M.C., Fujimoto, Y. & Hasegawa, S. *Phys. Rep.* **65**, 151 (1980).

Publications

Variations on a theme

from Jared Diamond

WE SCIENTISTS have chosen a cruel profession. To advance, we must publish, but our best works are often rejected, published in mangled form, ignored, or criticized. We must publish a lot, but not by plagiarizing the work of others nor by recycling our own material. While chasing grants, claiming priority for discoveries, and currying academy memberships, we risk missing deadlines, being passed over for appointments, or getting fired. We suffer grievously from politics, and few of us get rich. Worst of all, only a short span of productivity remains to us from the time we complete professional training until our creative powers wane. How are our problems to be solved? On the occasion of the 300th anniversary of Bach's birth (21 March 1685), what perspective can be gained from the way great composers faced these problems?

Incomprehensibly to modern scientists, desperate to amass long lists of publications, Bach never bothered to publish his B minor mass, St Matthew and St John passions, and Brandenburg concerti, which rank today among the monuments of western culture. Those works survived in manuscripts; his great C minor fantasia and fugue survived in a single copy, supposedly rescued from a pile of wrapping paper in a Leipzig shop; but his St Mark passion and dozens of cantatas mentioned by his contemporaries disappeared.

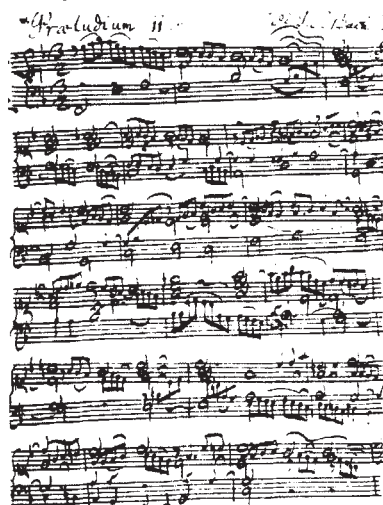
Other composers, eager to get their works published and decently produced, have encountered difficulties that are familiar to scientists. A few weeks before Schubert's death, four of his greatest works — the C major quintet and the last three piano sonatas — were rejected in one letter from the Leipzig publisher H.A. Probst. Beethoven's violin concerto was premiered without a rehearsal, and the soloist (one Franz Clement) interrupted it to play his own sonata, on one string with the violin turned upside down. Verdi could not complete *Aida* in time for the deadline (to celebrate the opening of the Suez Canal).

As for plagiarism, Mozart's requiem was commissioned by Count von Walsegg zu Stuppach, who liked to order works from professional composers and produce them as his own. The art of recycling works of one's own and others was honed to perfection by Bach and Handel. Of Bach's fourteen concerti for harpsichord, not more than two are original, while the Christmas and Easter oratorios were mostly lifted from his cantatas. Scientists have lost their jobs for far less extensive recycling.

Virtually all great composers have been exposed to vicious criticism. Berlioz despised Bach's music; Debussy, Fauré and Dukas walked out on Mahler's conducting of his second symphony; Weber viewed

Beethoven's seventh symphony as evidence of insanity; Brahms compared Bruckner's symphonies to endless serpents; and a critic likened Pasatieri's music to a "stream of perfumed urine". Compared with such metaphors¹, the invective of the most pugnacious modern biologists — the cladistic taxonomists and vicariance biogeographers — is unimaginative (for example, "Darwin preached from ignorance"²). Like the research of Mendel and Wegener, Ives's work found widespread approval only after his death.

Disappointments in the quest for positions and honours have loomed as large for musicians as for scientists. Through servile letters, Bach managed to wheedle the honorary title of royal court composer out



"... I am afraid we shall only be able to publish your manuscript if it is cut by ten bars..." (British Library).

of the Elector of Saxony. Other composers suffered crushing rejections. Few scientists take the loss of an NIH grant as hard as Tchaikovsky took the termination of his 14-year grant from the widow Nadezhda von Meck; he wrote bitterly that her name would be found carved in his heart when he died. To the end of his life, Brahms remained deeply wounded by the action of the Philharmonic Orchestra in Hamburg, his birthplace, in passing him over at the age of 30 as conductor. Schumann was compelled to resign as conductor at Düsseldorf, while the Vienna Opera fired Mahler in 1907 and Richard Strauss in 1924. A worse fate nearly befell Haydn, who as a teenager narrowly escaped castration by a choir director eager to preserve his soprano voice.

One might suppose a musician's career to be free of the problems of priority that are commonplace to scientists; a race to isolate the AIDS virus, yes, but surely not to compose the B minor mass? In fact, the shortage of suitable libretti has provoked

scrambles among opera composers rivaling that which pitted Pauling against Watson and Crick. Leoncavallo, who apparently wrote an operatic libretto based on a novel by Mürger and showed it to Puccini in 1893, was enraged to learn from Puccini over lunch in 1894 that he was busy at work on a rival libretto. Newspapers reported both composers' intentions that night, and the race was on. Puccini won by 15 months; he also wrote the better version of *La Bohème*.

Like great scientists, few great musicians become rich, and fewer still are shrewd enough to retain their riches. Bach's Brandenburg concerti were sold after his death for four gröschen apiece; Beethoven lost money on the premiere of his ninth symphony. Brahms eventually was able to sell his compositions for high fees but foolishly entrusted management of his money to his publisher Simrock, who lost heavily in stock market speculation.

Musicians have had even less success than scientists at using their talents for or against political causes. Prokofiev dutifully composed a cantata, *Hail to Stalin*, and was named People's Artist of the Soviet Union, but lived to see his works banned, like those of Lysenko's rival, the geneticist Vavilov. Shostakovich fell from official grace and regained it with his fifth symphony (subtitled "A Soviet Artist's Reply to Justified Criticism"), only to fall again.

Comparisons between scientists and musicians are especially instructive for psychologists studying how human creativity develops with age³⁻⁷. Creativity tends to flower and fade early in fields that require reasoning skills rather than varied knowledge, but late in fields requiring one to unite disparate data. Thus, pure mathematicians and molecular biologists peak in their twenties and thirties but evolutionary biologists peak much later. Darwin published *On the Origin of Species* when he was 50, and produced seven major books in the eleven years up to his death at 73, while Ernst Mayr recently completed his monumental *The Growth of Biological Thought* (Harvard University Press, 1982) at 78. Musicians, of course, are famous for precocity but it is less appreciated that most great composers, except Sibelius, continued to be creative until felled by illness or death. Composers whose music unites words, voice and instruments particularly tend to peak very late in life — they are the evolutionary biologists of the musical world. Haydn, Janacek, Lalo and Rameau produced their greatest operas, oratorios, or masses between the ages of 65 and 72. Verdi's masterpieces *Othello* and *Falstaff* were completed at 74 and 80, while Richard Strauss's poignant and richly orchestrated

Erratum

In the fifth paragraph of Ian Stewart's article "How bent is a knot?" (14 March, p.130), the sentence beginning "If $n = 1$ (M is a curve)" should have continued "then $4\sigma_1$ must be replaced by $2\sigma_1$. And if $n = 3$, $N = 4$, then σ_1 is replaced by..."

Four Last Songs, looking back on his long happy marriage and its impending dissolution by death, were written at 84. By contrast, I am unaware of any Nobel Prize awarded for work done after the age of 65.

Do the changes in the creative style of composers with age offer a message for scientists aware of shifts in their own creativity? The desire for recognition often drives scientists cruelly to demand of themselves a time scale of creative development that their subject matter does not permit. It is not easy for young evolutionary biologists to be patient, nor older molecular biologists to be satisfied with an enforced change of intellectual goals as they age. Many composers confronted with similar problems were able to find inner peace. Richard Strauss, like his equally precocious librettist Hugo von Hofmannsthal, remained creative to the end, but was well aware of, and adapted to, changes in his abilities. No longer able to spin out the long musical phrases that had marked the symphonic poems of his

youth, he turned instead to operas based on much shorter themes. The published correspondence of Hofmannsthal and Strauss⁸ will fascinate scientists concerned with mastering the problems of publishing or perishing as they grow older. They might also bear in mind that as his total output (especially of sacred cantatas) shrank with age, Bach concentrated on his two contrapuntal masterpieces, the *Musical Offering* and *Art of the Fugue*. □

1. Slonimsky, N. *Lexicon of Musical Injunctive*, 2nd edn (University of Washington, Seattle, 1975).
2. Nelson, G. & Rosen, D.E. (eds) *Vicariance Biogeography* (Columbia University, New York, 1980).
3. Dennis, W. *Science* 123, 716 (1956).
4. Roe, A. *Science* 150, 313 (1965).
5. Eiduson, B.T. in *Aging and Achievement* (ed. Taylor, C.W.) (Pennsylvania State University, University Park, 1968).
6. Eiduson, B.T. *J. Personality Assessment* 38, 405 (1974).
7. Goulet, L.R. & Baltes, P.B. *Life-span Developmental Psychology* (Academic, New York, 1970).
8. R. Strauss — H. von Hofmannsthal *Briefwechsel* (eds Strauss, F. & Strauss, A.) (Atlantis, Zurich, 1952).

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Nuclear structure

Taken with a grain of salt

from Virginia A. Zakian

THE cytoplasm of eukaryotic cells is permeated by a highly-ordered subcellular skeleton of filaments and membranes. Even non-structural cytoplasmic components such as glycolytic enzymes are firmly positioned within this three-dimensional array. It would not, therefore, be surprising if the nucleus possessed an organizing skeleton required for the proper positioning of sequences important for replication, transcription, and chromosome integrity. Indeed the appearance of chromosomes at cell division as well as recent studies on polytene chromosomes in *Drosophila* interphase nuclei¹ provide cytological evidence for nuclear order. A variety of biochemical studies also support this view, including the isolation by Laemmli and co-workers of what they call the nuclear scaffold²⁻⁵. In a recent paper, this group has explored the nature of DNA sequences associated with the scaffold in *Drosophila* cells in interphase and asked whether these associations change as a function of gene transcription: their answer is provocative.

It is still a matter of controversy whether a nuclear substructure exists at all. Scepticism centres on the methods used to prepare material suitable for biochemical analysis: because there is no assay for structural integrity, it is difficult to prove that interactions found in intact nuclei are maintained during isolation. Of the different procedures developed to probe nuclear substructure, the isolation of the nuclear matrix is the most widely used. The matrix is defined as the largely proteinaceous structure which remains after isolated nuclei are extracted with non-ionic

detergents and high-salt (2 M NaCl) and then digested with DNase⁶. Prior to the digestion, chromosomes are attached to the matrix at multiple sites such that the DNA is organized into topologically-independent, supercoiled domains⁷. After DNase digestion, the matrix retains the approximate shape of the nucleus and exhibits three major ultrastructural features: an outer lamina, a residual nucleolus, and a fibrillar network.

There is little consensus as to the nature of the DNA sequences involved in attachment to the matrix: some reports indicate that it is a random subset of the genome whereas others find an enrichment for repetitive sequences or specific genes. But a number of studies in a variety of systems suggest that the matrix provides a structural support for DNA replication, in that newly synthesized DNA is tightly associated with the matrix (see, for example, ref. 7). Moreover, many workers find that structural genes are preferentially associated with the matrix in cell types in which the gene is actively transcribed (see, for example, ref. 8). For some investigators these data are sufficient to indicate that the matrix serves a crucial role in organizing nuclear events; for others, they are artefacts of isolation, in that exposure of nuclei to high salt could induce precipitation of proteins or transcription complexes onto the matrix as well as allow sliding of DNA attachment sites.

Although the relationship between the nuclear matrix and Laemmli's nuclear scaffold is not clear, they share certain structural properties: for example, a subset of

nuclear proteins is common to both and both include the peripheral nuclear lamina. The scaffold is prepared from the metaphase chromosomes of mammalian cells by the depletion of their histones and most other nuclear proteins, leaving a proteinaceous axial structure which retains the approximate shape of the original chromosome and is surrounded by a halo of DNA loops². A rapidly-sedimenting structure with similar biochemical properties has been isolated from interphase cells³. The scaffold from both mitotic and interphase cells is sensitive to disruption by chelation and is believed to be held together by metalloprotein interactions^{4,5}.

For their recent investigation of the scaffold-associated DNA sequences in *Drosophila* interphase cells, Laemmli's group has developed a gentle, low-salt procedure of nuclear lysis. Isolated nuclei are extracted with buffers containing spermidine and lithium di-iodosalicylate (LIS), a salt with detergent-like properties which can be efficiently removed after use. These histone-depleted nuclei are washed, pelleted, and digested with restriction enzymes which solubilize about 70 per cent of the DNA. Both the solubilized DNA and the DNA in the residual nuclear scaffold are then assayed for the presence of specific sequences, including fragments of histone and heat-shock genes.

When fragments derived from the five-kilobase histone gene repeat unit are examined, one fragment is found exclusively with the scaffold whereas all the other fragments are found primarily in the supernatant. The scaffold-bound fragment is rich in adenine and thymine (A + T) and is derived from a non-transcribed 'spacer' sequence upstream of the histone 1 gene. Likewise an A + T-rich fragment from the non-transcribed spacer of the *hsp70* heat-shock genes is scaffold-associated. Moreover, this association is independent of the transcriptional state of the *hsp70* genes. Finally, many copies of a middle-repetitive A + T-rich element, including a copy found upstream of the *hsp70* gene, are scaffold-bound. These results suggest that the DNA loops of interphase nuclei are maintained through sequence-specific associations with scaffold proteins and that these associations do not change as a function of transcriptional activity. None of the specific associations are maintained if LIS-extracted nuclei are treated with 2 M NaCl after digestion with restriction enzymes. One interpretation is that a high concentration of salt disrupts the specific DNA-protein interactions in the scaffold; at the

1. Mathog, D., Hochstrasser, M., Gruenbaum, Y., Saumweber, H. & Sedet, J. *Nature* 308, 414 (1984).
2. Paulson, J.R. & Laemmli, U.K. *Cell* 12, 817 (1977).
3. Lebkowski, J.S. & Laemmli, U.K. *J. mol. Biol.* 156, 309 (1982).
4. Lewis, C.D. & Laemmli, U.K. *Cell* 29, 171 (1982).
5. Mirkovitch, J., Mirault, M.-E. & Laemmli, U.K. *Cell* 39, 223 (1984).
6. Berezney, R. & Coffey, D.S. *J. Cell Biol.* 73, 616 (1977).
7. Vogelstein, B., Pardoll, D.M. & Coffey, D.S. *Cell* 22, 79 (1980).
8. Ciejek, E.M., Tsai, M.-J. & O'Malley, B.W. *Nature* 306, 607 (1983).

same time it induces non-specific associations of active-gene regions through precipitation of transcription complexes⁵.

These results are provocative: although they support the hypothesis that the nucleus is highly ordered, they raise doubts about the validity of methods widely used to analyse nuclear structure. However, in the absence of an accepted assay for structural integrity, this report must be evaluated with caution. It is possible that the LIS-extraction procedure introduces its own artefacts: for example, it might induce precipitation of A + T-rich sequences on to the residual scaffold. It is also possible that there are different classes of DNA-protein associations in intact nuclei, only some of which are transcription-dependent and only some of which are maintained through different isolation procedures. For example,

it is easy to imagine that DNA interactions with the peripheral lamina will display a different sensitivity to extraction conditions than do interactions with the internal fibrillar network.

The argument against high-salt methods for the study of nuclear structure will be strengthened if others obtain similar results in different systems. Convincing proof of the importance of a given nuclear substructure may ultimately require the identification of mutations in its components; investigations in a lower eukaryote, amenable to genetic analysis, may be the most profitable way to establish functional basis for nuclear structure. □

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Magnetochemistry

How many atoms make a metal?

from D. Michael P. Mingos

MAN'S ability to recognize and exploit the characteristic properties of metals has been an important part of his technological development, although the magnetic and electrical properties of metals have only been fully utilized in the past hundred years. The scientific interpretation of these characteristics is based on a band-theory model, which raises the question of whether the characteristic properties of a metal are still displayed by the molecular clusters containing as few as 10–100 metal atoms that have been synthesized in recent years. The paper by D.C. Johnson *et al.* on page 231 of this issue examines transition metal cluster compounds and suggests that certain magnetic properties that they are characteristic of the bulk metal begin to appear when as few as ten metal atoms are present.

The band-theory model describes a metal as an infinite and regular three-dimensional array of atoms where electrons are no longer localized but are shared between the constituents, which are thus able to respond cooperatively to external forces. Ideally, studies on emergent metallic properties should be carried out on small-diameter particles (5–100 Å) but these pose severe experimental and technical problems. First, such particles do not all contain the same number of metal atoms but have a statistical distribution of sizes. Second, to avoid contamination by oxide species, it is necessary to study them under high vacuum conditions.

The strategy adopted by Johnson and his colleagues avoids these difficulties by investigating molecular cluster compounds of osmium, composed of 3–10 atoms, that are held together at distances comparable to those found in the bulk metal. These clusters have a small chunk of bulk metal at their centre, but each metal atom is also

bonded to carbon monoxide molecules, which stabilize the cluster and provide a protective sheath to stop the cluster aggregating into particles of bulk metal. Such cluster compounds have been synthesized in large numbers in recent years, particularly by research groups in Cambridge and Milan, and have been structurally characterized by single-crystal X-ray techniques. As a result, well-characterized compounds with fixed numbers of metal atoms and high purity are now available in reasonable quantities. They are generally air-stable and highly-coloured crystalline solids.

Johnson *et al.* have measured the magnetic properties of these compounds using a high sensitivity computer-interfaced Faraday magnetic balance in the temperature range 1.5–300 K. The compounds show a temperature-independent paramagnetic susceptibility, which is not surprising since, in a formal sense, all the electrons are paired up in metal-metal bonds. The observed paramagnetism arises

from excited states that are not thermally accessible. The interesting aspect of the results arises from a positive correlation between the observed paramagnetism and the number of metal atoms in the cluster. They argue that the paramagnetism increases in this sequential fashion because molecular electronic states begin to develop into the band and continuum states that are characteristic of the bulk metal. The number and energies of the excited states increase with the number of metal atoms in the cluster, as demonstrated by a red shift in the electronic spectra, and consequently they make a larger contribution to the observed paramagnetism. This leads them to predict that transition-metal cluster compounds containing more metal atoms may show very interesting electronic properties that arise from a polarization catastrophe within the clusters themselves.

The authors are more equivocal in their answer to the fundamental question of how many atoms are needed in a cluster to mimic effectively the bulk properties of a metal. This depends on the particular property being measured. Transport properties, such as electrical conductivity, require complete ionization of electrons from the parent site — a process which might require 0.1–10 eV. — before metallic character can be detected. Magnetic properties, in contrast, are observed for clusters with quite small numbers of metal atoms, because modification of the intra- and inter-unit exchange energies requires only about 10⁻³ eV.

An industrial chemist is more likely to want to know how big the cluster need be before it performs effectively and selectively as a catalyst in a chemical reaction, a question that cannot be answered by this type of magnetic measurement. Chemists have, however, now synthesized molecular clusters with up to forty metal atoms, so no doubt they will soon be able to design suitable experiments to answer this question. □

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Satellite oceanography

Radar images of the sea bed

from J.F.T. Zimmerman

SINCE G.P. De Loor first observed sand-wave-like features in radar images of the sea surface^{1,2}, the SEASAT satellite has produced many examples of the radar imaging of bottom topographic features^{3,4}—as in the accompanying figure. Whether taken from side-looking airborne real-aperture radar (RAR)^{1,2} or satellite-borne synthetic radar (SAR)^{3,4}, at first sight a sea bottom topographic image is something of a mystery. In a recent paper⁵ (see also page 245 of this issue⁶),

Alpers and Hennings have given a firm theoretical foundation to the most likely solution of the mystery.

The images may seem to be mysterious for two reasons. First, radar waves do not penetrate into the sea for more than a centimetre, so no direct reflectance from the bottom is possible in waters which may be up to some decametres in depth. Second, since radar backscattering from the sea surface occurs primarily by first-order Bragg-scattering⁷ (on account of short surface

windwaves with a wavelength half that of the horizontal component of the radar wavelength — of the order of several centimetres to decimetres) and since, as a rule of thumb, surface waves do not 'feel' the bottom when the water depth exceeds half the wavelength, the Bragg-resonant surface waves cannot form an image of the bottom topography in any direct way. So, from the outset it has been clear that it is necessary to find at least one extra link, connecting modulations in the amplitude of the scattering surface waves to fluctuations in water depth. Although several hypotheses have been raised for this missing link, one has always been a strong candidate. It invokes the modulation of the (tidal) current velocity field by the bottom topography, the resulting spatial variability of the current velocity field in turn modulating the energy

ified for topographic length scales that are small compared with the tidal wavelength. Thus any tidal current with a velocity component in the direction of the local bottom slope produces divergences or convergences in the velocity field. As Alpers and Hennings show, these velocity gradients can produce images of the slope of the sea bed (rather than the depth itself) in two ways, one of which applies only to SAR and the other to both SAR and RAR.

The first mechanism is, in fact, an artefact of the SAR imaging process, called 'velocity bunching'. In the absence of currents, the azimuthal (along-flight) position of a SAR target is determined by the vanishing of the Doppler frequency shift when the target is at a right angle to the flight direction. When the target moves with a current having a component in the radial

and an energy exchange with the current-velocity field due to the wave-radiation stress working against the strain-rate of the velocity field. This causes the wave energy density to increase when the current velocity increases in the direction of propagation, and *vice versa*.

This, in itself, would provide a mechanism of imaging but a second process also plays an important part. The short Bragg-resonant surface waves are located in that part of the wavenumber spectrum that is called 'saturation range'. This denotes the quasi-steady state in which a delicate, but poorly understood, balance between wind-forcing, nonlinear interaction among different wave components and dissipation by wave breaking maintains a spectrum in which the energy density falls with the fourth power of the wave number. So, if the straining of the velocity field perturbs the energy level of a certain wave component in the saturation range, one may expect this component to relax to its original value either by enhanced breaking (in the case of oversaturation) or by feeding upon the wind (in the case of undersaturation). Alpers and Hennings choose to parameterize this process by simple linear relaxation, characterized by a relaxation time scale that is of the order of a minute at most, and is short compared with the time (of the order of 5–60 minutes) that the waves need to travel over a typical bottom surface. The perturbation in energy density level for a specific location is given by a simple balance between the forcing due to the strain-rate of the velocity field and the relaxation process, and so it is proportional to the velocity gradient. This, in turn, is proportional to the bottom slope (divided by the square of the depth), so the energy density of the scattered waves — and thus the intensity of the radar signal — is largest on the downstream side of the ridge and smallest on the upstream side. As a consequence, when the current flows in opposite directions during the ebb and flood of the tide, a sign reversal of the image intensity should occur, which is indeed observed in RAR images^{1,2}. This, together with the estimated relative strength of image-contrasts (20–30%), represents a firm corroboration of the theory.

Evidently, the theory also applies to the imaging of any other phenomenon that causes velocity divergences or convergences at the ocean surface — for instance, internal waves, trapped at strong vertical density gradients in the ocean interior, produce an undetectable vertical displacement at the ocean surface. Such waves have shown up in radar images and again can be explained by the stretched formalism⁶.

Successful as the theory may look, it rests on some drastic simplifications, in particular on the form and strength of the relaxation mechanism. This is clearly brought out by comparing the paper of Alpers and Hennings with that of Phillips¹², which runs more or less along the same lines, albeit without a discussion

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The Dutch Wadden Sea and IJssel Lake, separated by an enclosure dyke, as viewed by SEASAT-SAR. Whereas the Wadden Sea has strong tidal currents, the dyke excludes them from the IJssel Lake. The tidal channel system in the Wadden Sea is clearly visible but the relict tidal topography in the IJssel Lake is not visible due to the absence of tidal currents. (Courtesy National Aerospace Laboratory, Amsterdam).

density of the relevant part of the surface wave spectrum⁸. It is this mechanism that has now been given a firm theoretical basis by Alpers and Hennings⁵.

Their contribution arises from a synthesis of theoretical results already obtained for the various interactions between electromagnetic (radar) waves, surface waves, tidal currents and bottom topography. For the modulation of tidal currents by the sea bed, the simplest possible approach is followed, resting on two assumptions. One is that there is divergence-free volume transport, which gives an inverse relationship between the cross-isobath velocity component and the water depth; the other is that the along-isobath velocity component is independent of position. Although the latter may be an oversimplification in the light of velocity-current interaction theories^{9,10}, the former is certainly just-

direction of the radar beam, an additional Doppler shift is produced so that the target is given a false position in the image in the azimuthal direction. In the presence of gradients in the radial velocity, targets are differently misplaced causing the otherwise homogeneous grey tone to show areas of dark and light colouring according to the sign of the velocity gradient, which in turn is related to the bottom slope.

Alpers and Hennings estimate the strength of this process to be nearly an order of magnitude weaker than the second mechanism, which involves the hydrodynamic interaction between the Bragg-resonant surface waves and the tidal velocity field. Two ingredients of surface-wave theory¹¹ are relevant to this mechanism. Waves propagating at the sea surface in a nonhomogeneous horizontal current-velocity field experience both refractive effects

of topography-current interactions. The basic difference is that Phillips uses a more sophisticated parameterization of the relaxation. He stresses the fact that return to equilibrium by wave breaking in the case of oversaturation can be a more vigorous process than feeding upon the wind in the case of undersaturation. This may lead to strong asymmetry in the imaging process even in cases where the imaged current-velocity field has a symmetrical shape itself. Under certain conditions this leads to remarkable deviations from the results of Alpers and Hennings. For instance, when a local divergence of the velocity field decreases the wave-energy density, return to equilibrium may be so slow that the actual balance is not between straining and relaxation but between straining and advection. In this case, it is not the gradient of the current but the current velocity itself that is imaged by the radar (not the slope of the bottom but its inverse depth). For convergence, on the other hand, the relaxation of oversaturation by wave breaking may happen so quickly that excessively large strain rates are necessary to produce sufficient image contrast.

Although observations seem to support

Alpers and Hennings, it is no surprise that careful observations of the relevant physical parameters in tidal areas are recommended in both papers to settle the dispute over the relaxation time scale. Perhaps this is a pious wish — but both off the Dutch coast in the southern North Sea and at the Nantucket shoals near Massachusetts¹³, experiments have been set up to tackle these questions. □

1. De Loor, G.P. & Brunsfeld van Hulten, H.W. *Boundary-layer Meth.* 13, 119 (1978).
2. De Loor, G.P. *IEEE J. Oceanic Engng.* OE-6, 124 (1981).
3. Lodge, D.W.S. in *Satellite Microwave Remote Sensing* (ed. Allan, T.D.) 246 (Ellis Harwood, Chichester, 1983).
4. Kenyon, N.H. in *Satellite Microwave Remote Sensing* (ed. Allan, T.D.) 261 (Ellis Harwood, Chichester, 1983).
5. Alpers, W. & Hennings, I. *J. geophys. Res.* 89, 10529 (1984).
6. Alpers, W. *Nature* 314, 245 (1985).
7. Valenzuela, G.R. *Boundary-layer Meth.* 13, 61 (1978).
8. Valenzuela, G.R., Chen, D.T., Garrett, W.D. & Kaiser, J.A.C. *Nature* 303, 687 (1983).
9. Huthnance, J.M. *Estuar. coast. mar. Sci.* 1, 89 (1973).
10. Zimmerman, J.T.F. *J. mar. Res.* 38, 601 (1980).
11. Phillips, O.M. *The Dynamics of the Upper Ocean* 2nd edn (Cambridge University Press, 1977).
12. Phillips, O.M. *J. phys. Oceanogr.* 14, 1425 (1984).
13. Valenzuela, G.R., Chen, D.T., Garrett, W.D. & Kaiser, J.A.C. *EOS Trans. Am. Geophys. Un.* 64, 618 (1983).

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Blood volume regulation

On the right lines at last?

from W.E. Balfour

A WELCOME and secure step in our understanding of body fluid regulation is reported by Lang and his colleagues from Heidelberg on page 264 of this issue¹. Their experiments on the rat heart show that a peptide is released from the atria in response to stretching in the isolated heart, or to expansion of blood volume in the intact animal. Since the peptide is one of the recently characterized atrial natriuretic factors that regulate fluid excretion by the kidney, the fluid regulation control loop between heart and kidney is confirmed.

The idea that blood volume is hormonally controlled is not new², nor is the possibility that low pressure elements of the heart can sense the fullness of the circulation³. What is new is that the heart atria contain secretory granules, the contents of which are released in response to stretch rather than depending on an automatic reflex. Lang *et al.* have shown these effects by using a radioimmunoassay developed after the atrial peptides had been isolated and synthesized⁴. The atria contain an immunoreactive molecule which is larger than that secreted in response to a stretch. Lang's group stretched the atria in two ways: by small increases in perfusion pressure in the vena cava of the isolated heart, and by expanding the blood volume by injection of graded quantities of saline in the intact animal. In each case, the response is a graded release of relatively large amounts of atrial peptide, which has

a short half-life.

Unfortunately, the renal response was not measured by Lang *et al.* There are, however, two groups of experiments which do include a renal response; they are focussed on the left rather than the right atrium, but few would dispute that blood volume expansion raises the pressure in both atria. Linden's⁵ experiments dissect the functional components of the response to distension of the left atrium, brought about by inflation of balloons, in anaesthetized dogs. The renal response, requiring an intact vagus nerve, is an increase in water excretion, which, in the short term of these experiments, does not result in any substantial correction of the plasma volume, the expansion of which is simulated by the balloon. Ledsome⁶ has recorded similar reversible changes together with a fall in plasma vasopressin concentration, a result which is consistent with the hypothesis that atrial distension leads to a water diuresis.

On the other hand, Kaczmarczyk *et al.*⁷, working with surgically-prepared, yet conscious, trained and confident dogs, have used three stimuli to raise the atrial pressure and thus to effect a brisk increase in the excretion of both salt and water. Two of the stimuli, intravenous saline loading and ingestion of a sodium-containing meal, are just as effective after denervation of the heart, whereas stimulation by mitral stenosis, which raises only the left atrial

pressure, is not effective after denervation. It is of particular interest that all of these effective stimuli are associated with a fall in the concentration of plasma renin. Only the stimulus of mitral stenosis is prevented by denervation of the heart.

More recently, Lee *et al.*⁸, also working with conscious dogs, have shown that partial constriction of the ascending aorta fails to provoke the release of renin, even though the downstream fall in arterial pressure is similar to that which would be effective if the descending aorta were constricted. Constriction of the ascending aorta raises the left atrial pressure which inhibits renin release, probably through a hormonal mechanism. Thus, in these manoeuvres, increasing atrial stretch reduces the plasma concentrations of both renin and, it seems, vasopressin. The crucial question is whether these changes are causally related to the renal elimination of sodium and water in the proportions required for volume correction.

There have been few studies describing how atrial natriuretic factor causes natriuresis and diuresis. The reported potency of the peptide varies widely. This is probably related to whether the animals are conscious or anaesthetized, hydrated or dehydrated, sodium deficient or replete. Much less material is required if the renal artery is the route of administration but, so far, only anaesthetized animals have been used.

Two renal effects of atrial natriuretic factors — vascular and renin-reducing — have so far been recognized. The first is a specific reduction in renal vascular resistance⁹, associated with which is an increase in renal papillary plasma flow¹⁰ as high as that observed in rats congenitally lacking vasopressin (Brattleboro rats)¹¹. Such increases in renal medullary plasma flow would be expected to dissipate (as they do in Brattleboro rats) the medullary osmotic gradient, which is a prerequisite for the excretion of a copious isotonic urine. Brattleboro rats, or rats whose blood vasopressin concentration is low from having recently drunk water, excrete an isotonic urine at rates as high as one ml min⁻¹ in response to maintained blood volume expansion¹².

Urine flows of one ml min⁻¹ represent such a large recovery of the glomerular filtrate that they can be achieved only at the expense of a reduction in the normal level of fluid reabsorption in the proximal tubule. It is possible that the second action of atrial natriuretic factor — reducing renin secretion — is important here. Proximal-tubule puncture studies in rats whose renin-generated angiotensin II levels have been reduced by captopril, show a marked reduction in sodium reabsorption¹³, and Shirley¹⁴ has argued that the increased sodium excretion seen on captopril treatment of anaesthetized Brattleboro rats is likely to be a proximal-tubule effect.

The distal tubule has a much smaller role to play in the control of natriuresis,

particularly in short-term volume control, although it can become important as a fine control mechanism in the longer term. It is interesting that the angiotensin II-stimulated release of aldosterone from adrenal glomerulosa cells¹⁵⁻¹⁷ can be inhibited by physiological amounts of the atrial factor and that avid receptors for the atrial peptide have been found in kidney tubules in smooth muscle of the vasculature and in glomerulosa cells¹⁸.

It is necessary to ask whether the fall of both plasma renin and of vasopressin exerts a regulatory or permissive role on the excretion of salt and water. This will not be answered until there have been measurements matching the observed changes in the concentration of atrial factor in the plasma to the different sensitivities in the control of the secretion of these two hormones. There is a long way to go before we can emulate Verney's observation¹⁹, in the

allied field of water balance, that a one per cent rise in the osmotic pressure of blood going to the brain is sufficient to produce a maximal antidiuresis. □

1. Lang, R.E. *et al.* *Nature* **314**, 264 (1985).
2. de Wardener, H.E. *Clin. Sci. mol. Med.* **53**, 1 (1977).
3. Henry, J.P. *et al.* *Circ. Res.* **4**, 85 (1956).
4. Atlas, S.A. *et al.* *Nature* **309**, 717 (1984).
5. Linden, R.J. & Kappagoda, C.T. *Atrial Receptors* (Cambridge University Press, 1982).
6. Ledsome, J.R. *et al.* *J. Physiol. Lond.* **338**, 413, (1983).
7. Kaczmarczyk, G. *et al.* *Pflügers Arch.* **390**, 125 (1981).
8. Lee, M.E. *et al.* *Fed. Proc.* **43**, 995 (1984).
9. Oshima, T. *et al.* *Circ. Res.* **54**, 612 (1984).
10. Borenstein, H.B. *et al.* *J. Physiol. Lond.* **334**, 133 (1983).
11. Bayle, F. *et al.* *Pflügers Arch.* **394**, 211 (1982).
12. Balfour, W.E. *et al.* *J. Physiol. Lond.* **332**, 94P (1982).
13. Harris, P.J. *et al.* *J. Physiol. Lond.* **327**, 93P (1982).
14. Shirley, D.G. *et al.* *J. Physiol. Lond.* **346**, 107P (1984).
15. Kudo, T. *et al.* *Nature* **312**, 756 (1984).
16. Chartier, L. *et al.* *Endocrinology* **115**, 2026 (1984).
17. DeLean, A. *et al.* *Endocrinology* **115**, 1636 (1984).
18. Napier, M.A. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **81**, 5941 (1984).
19. Verney, E.B. *Proc. R. Soc. B135*, 25 (1947).

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Solid state dynamics

Unsolved problems of creep

from J. Weertman

ON page 255 of this issue Doake and Wolff¹ bring new information to a very intriguing, unresolved problem in high temperature creep of crystalline solids. The outcome of their examination of creep flow of ice in polar ice sheets is of importance not only to glaciers but to the deformation of the Earth's mantle and, of course, to the understanding of the creep process itself.

The origin of the problem is the old observation by Harper and Dorn² of a creep rate that is proportional to the applied stress (Newtonian creep) in aluminium at relatively low stresses, in conditions such that the creep should be produced by dislocation motion and so is expected to be proportional to a power of the applied stress. This expectation arises from the fact that creep rate is proportional to the product of the dislocation density and the average dislocation velocity. The former quantity, in steady state, is proportional to the stress squared and the latter to the stress. Hence the creep rate is proportional to the cube power of the stress. (Higher powers are also possible^{3,4}.)

It was easy to dismiss the original Harper-Dorn experiment by claiming that steady-state creep had not been attained. The initial dislocation density in material that shows decelerating creep before steady-state creep is reached is higher than when steady-state conditions prevail. If the creep rate is measured only in a small initial strain region, the dislocation density may be approximately a constant during the course of creep experiments. Hence the creep rate falsely seems to be proportional to stress. Very recently, Soliman and Mohamed⁵ have shown experimentally that this applies to the original Harper-Dorn experiment and to more recent experiments that were

not extended to large strains. It does not, however, apply to experiments in which Harper-Dorn creep has been measured in metals with large strains⁶.

Blacic and I⁷ recently offered an explanation of Harper-Dorn creep that has yet to be tested experimentally. We pointed out that the temperature cycling that is inevitable in the usual high-temperature creep tests is sufficient to produce large chemical stresses on the dislocations by periodically altering the value of the equilibrium-point defect density. Hence the dislocation density can be controlled by the chemical stress rather than by the applied mechanical stress. The average dislocation velocity remains proportional to the applied mechanical stress and the creep is Newtonian. This explanation, that Harper-Dorn creep is an artefact of temperature cycling, does not work for Harper-Dorn creep of ice in glaciers and in polar ice sheets, because seasonal temperature fluctuations are effectively removed at depths greater than about ten metres. (This has also been pointed out by Lliboutry and Duval⁸.)

Nor, for the same reason, does it work for Newtonian creep in the Earth's mantle. Glacial rebound data and the anomalous motion of satellites orbiting the Earth are very nicely accounted for by analyses of mantle deformation that are based on Newtonian creep for the mantle^{9,10}. Moreover, Harper-Dorn creep has been observed by Poirier *et al.*^{11,12} in a mineral analogous to one of the presumed major minerals of the lower mantle.

If Harper-Dorn creep is real, and not just a peculiar experimental artefact, life is made easier for modellers of both mantle flow and glaciers. They can use the simpler linear constitutive law in their

work. But the rub is that transient creep still cannot be ruled out as the explanation of the evidence for mantle and glacier Harper-Dorn creep. The creep strains that are involved in the analysis of both glacial rebound and anomalous satellite motion are generally quite small. Hence it is still possible to dismiss the application of these results to convection currents in the mantle, where clearly the strains are quite large.

As pointed out by Doake and Wolff¹, the measurements they analysed may also involve non-steady-state creep. The tilt of boreholes gives the creep rate as a function of depth, but the shear stress also increases with depth. To have steady-state creep, the stress must remain constant. Their results can be accounted for either if ice at moderate stresses is indeed Newtonian or if transient creep effects make it appear to be Newtonian although it is not. The same is true of the evidence that indicates Newtonian mantle creep. To help resolve this problem, it would be well worth obtaining the spreading rate of the Ward Hunt Ice Shelf in the Canadian Arctic. This ice shelf is much thinner than the Antarctic shelves considered by Doake and Wolff, and should give another, large creep-strain datum point at a much lower stress level. If ice is Newtonian and if it can be shown that temperature cycles do not account for Harper-Dorn creep in laboratory experiments, the very interesting phenomenon of creep in crystalline solids will remain to be solved. □

1. Doake, C.S.M. & Wolff, E.W. *Nature* **314**, 255 (1985).
2. Dorn, J.E. & Harper, J.G. *Acta metall.* **5**, 654 (1957).
3. Weertman, J. in *Proc. 2nd. int. Conf. Creep and Fracture of Engng. Mater. and Struct.* (eds Wilshire, B. & Owen, D.R.J.) 1 (Pineridge Press, Swansea, Wales, 1984).
4. Sherby, O.D. & Weertman, J. *Acta metall.* **27**, 387 (1979).
5. Soliman, M.S. & Mohamed, F.A. *Mater. Sci. and Engng.* **68**, L23 (1985).
6. Mohamed, F.A. & Ginter, T.J. *Acta metall.* **30**, 1869 (1982).
7. Weertman, J. & Blacic, J. *Geophys. Res. Lett.* **11**, 117 (1984).
8. Lliboutry, L. & Duval, P. *Annales Geophysicae* (in the press).
9. Peltier, R. *Advances in Geophysics* **24**, 1 (1982).
10. Lambeck, K. *Nature News and Views* **309**, 584 (1984).
11. Cahn, R.W. *Nature News and Views* **308**, 493 (1984).
12. Poirier, J.P., Peyronneau, J., Gesland, J.Y. & Barbec, G. *Phys. Earth planet. Int.* **32**, 273 (1983).

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Scanning electron micrograph ($\times 975$) of basalt glass after leaching in NaCl solution at 200°C for 30 days — a model for the stability of nuclear waste glasses. See page 252.

Protein engineering

Hydrogen bonds in active sites

from John Galloway

SINCE the sceptical reception of Latimer and Rodebush's suggestion in 1920 that they hold water molecules together, hydrogen bonds have come a long way. They are now widely believed to play the definitive role in determining molecular specificity. Hence the interest of protein engineers in hydrogen bonds and the paper on page 235 of this issue in which Alan Fersht and colleagues analyse, by means of systematically altering the amino acids of the active site of an enzyme, the hydrogen bonds that determine substrate specificity.

Full realization of the potential of protein engineering is some way off. The chief problem is our inexact understanding of the energetics of the three-dimensional structures of proteins and of the relationships between those structures and the proteins' functions. It is well enough established that the sequence of amino acids uniquely defines a protein's three-dimensional structure and, in doing so, confers on it a capacity to interact functionally with perhaps only one other molecule. Driven to the molecular level, the study of biological specificity thus becomes the detailed analysis of this structural and functional idiosyncrasy and the mechanisms by which it is achieved.

Without a detailed understanding of the way proteins are put together, it is inconceivable that new proteins can be designed or old proteins given new roles. But that understanding is hard to win in the degree of detail needed, and the capacity to determine structure and function by protein crystallography still far outstrips any attempt to predict it from amino-acid sequence. It may become possible to mimic protein folding by analogue means but otherwise we are left with energy calculations which are not easy. Although, among other things, fair calculations can be done 'in vacuo', proteins fold in water, which probably mediates all interactions of any interest. Since it is now possible to change any residue in a protein by site-directed mutagenesis of its gene, the present compromise is to study empirically the structural and energetic changes associated with small changes in amino-acid sequences.

For Fersht *et al.*, the protein under study is the enzyme tyrosyl-transfer RNA synthetase which activates tyrosine, forming a complex between the enzyme and the intermediate tyrosyl-adenylate, before transferring the amino acid to its transfer RNA. (The enzyme is of no apparent medical or commercial interest.) The power of the approach lies in combining site-directed mutagenesis both with enzyme kinetics, which gives accurate estimates of changes in binding energies, and with high-resolution crystallography, which gives the

detailed structural changes. (Without high-resolution crystallography the technique can still be used, but merely as a probe of the role of residues in catalysis, for instance.) A logistical difficulty that such combined methodology usually has to face is that the techniques of mutagenesis and kinetics are quick to yield results, but protein crystallography tends to lag behind. It is striking that this is not the case for the tyrosyl-transfer RNA synthetase studies, presumably in part because of growing familiarity with its structure.

The most noteworthy of a number of findings described by Fersht *et al.* is that a hydrogen bond involving a side chain with a net charge on it is stronger — by up to a factor of about six — than a hydrogen bond with no such involvement. Indeed by the time the difference in energy is properly weighted by a Boltzmann exponential factor, a 'charged' hydrogen bond may well contribute one to two orders of mag-

nitude more to specificity than an 'uncharged' one. That is useful to know in building up a database of interactions and it also reminds us of something that is often forgotten — hydrogen bonds are to a large extent simply a convenient way of looking at a part of the overall spectrum of electrostatic interactions between molecules or parts of molecules. A convenient way of expressing such an interaction is in terms of a multipole expansion

$$\frac{e_1 e_2}{r_{12}} + \frac{e_1 \mu_2 + e_2 \mu_1}{r_{12}^2} + \frac{\mu_1 \mu_2}{r_{12}^3} + \dots$$

where e represents net charge and μ a dipole and 1, 2 refer to the interacting groups. The leading term in computing a hydrogen bond between neutral groups — like water molecules — is the third one. But if one group has a net charge, then the second term will lead and typically will be a good deal bigger. Since the problem is complicated by the presence of strongly dipolar water molecules, it would not have been very surprising had the effect of the charged hydrogen bond not shown up. That it has done, is well worth noting. □

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Ecology

Competition in imaginary worlds

from Paul H. Harvey and Robert M. May

TO WHAT extent does competition influence the distribution of species among the islands of an archipelago? This seemingly straightforward question cannot easily be answered despite scores of studies using a variety of techniques to analyse the distributions of many plant and animal species from several groups of islands¹. No consistent patterns emerge. Indeed, various authors working on the same data set often reach different conclusions because they have used different assumptions in their statistical models. A new approach by Colwell and Winkler², using a computer to generate a myriad of imaginary worlds, helps us to understand the limitations of conventional analyses for detecting the factors that actually govern the distribution of species in archipelagos.

The essential problem is that each archipelago is unique, with its plant and animal species put in place by the slow workings of evolutionary processes. The logically appealing techniques of The Scientific Method — manipulative experiments with controls and replicates — are not easily applied in the real world. As emphasized by Simberloff, Strong and others at Florida State University³, one way of trying to tease out the processes underlying observed patterns of distributions of species is to compare these patterns with null models constructed by randomly reshuffling the species among the islands. But the effects

of competition may still inadvertently be built into the null models, which assume that competition is absent

Colwell and Winkler² shed light on these problems by generating imaginary archipelagos, each populated according to specified rules. Using the worlds they have created, they explore the extent to which conventional methods of analysis can detect the competitive or other factors that actually govern the distribution of species in their archipelagos.

They begin by generating phylogenies, using a simple simulation model designed by Raup and Gould⁴. As the hypothetical species evolve, their phenotypes change gradually so that, on average, closely related species are more like each other than are more distantly related species. For the purposes of the model, easily measurable characters such as beak length and width are chosen. This evolution takes place on a large land mass under the guidance of a computer program called GOD. The organisms then disperse to the islands of a nearby archipelago, their destinations determined by additional computer routines, summarized in the figure.

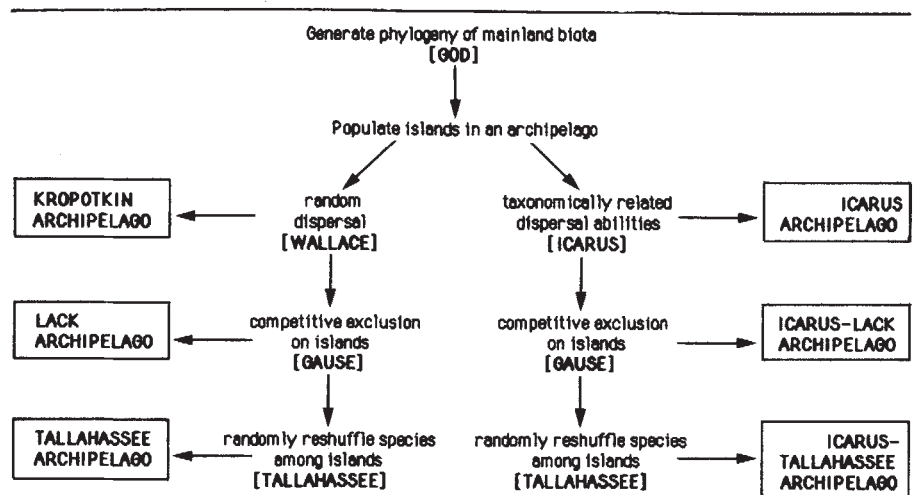
One routine allows random invasion of the different islands with subsequent interspecific competition determining which species establish successful populations on each island. When phenotypically similar species invade an

island, some are likely to become locally extinct as a result of competitive exclusion. An alternative routine allows random invasion of islands from mainland without subsequent competition. As competition occurs only in the first routine, the second makes a suitable null model for comparison. One recent empirical study uses exactly this method: Graves and Gotelli⁵ compare the actual avifaunas on land-bridge islands in the New World against the distribution patterns generated by sampling appropriate pools of mainland species.

But a different type of comparison is often made with real data. Species found on different islands are randomly redistributed among the same islands (by computer of course); the reassortment is then compared with the original distribution pattern³. Colwell and Winkler incorporate a computer routine that mimics this procedure. They find that the species distributions differ very little between the reassorted archipelago and the one on which competition has occurred, thus confirming that the effects of competition are indeed partly incorporated into the null model derived from the reassortment routine. Consequently a comparison that uses this procedure is less likely to detect the effects of competition than is Colwell and Winkler's first procedure^{6,7}.

Another factor, usually ignored, that leads to underestimating the influence of competition, is the varied abilities of different taxa to disperse^{6,8}. For example, kingfishers are more vagile than kiwis, and so are likely to be found on islands which kiwis have not reached. In a second series of comparisons, Colwell and Winkler allow dispersal abilities as well as morphological characteristics to evolve in the phylogenies generated by their computer. The result is that closely related species tend to end up on the same islands. Some species are extirpated when competition occurs, thus producing a species assemblage on each island that is taxonomically less biased than that of the original invaders. Because differential dispersal abilities produce the opposite pattern from competition if they are ignored when designing null models, the effects of competition are shown to be consistently underestimated.

Colwell and Winkler have also contributed to the comparisons of the dist-



Colwell and Winkler² generate a computer phylogeny using a routine called GOD, and distribute species among the islands of an archipelago according to rules specified by either WALLACE or ICARUS. The species on each island are then subjected to competition using GAUSE, to produce archipelagos with new species compositions. Finally the species on the post-competition archipelagos are redistributed among the islands using TALLAHASSEE. Recent analyses of real data sets search for the effects of competition by comparing the equivalent of the LACK and ICARUS-LACK ARCHIPELAGOS with the TALLAHASSEE and ICARUS-TALLAHASSEE ARCHIPELAGOS respectively. These comparisons consistently underestimate the effects of competition². More suitable nulls models against which to compare the LACK and ICARUS-LACK ARCHIPELAGOS are the KROPOTKIN and ICARUS ARCHIPELAGOS respectively.

tributions of species that are not likely to be in competition, the subject of several recent discussions⁹⁻¹¹. Classic cases in the literature include searching for competitive exclusion between owls and hummingbirds or between ducks and warblers¹². The simulation study helps to place this problem in context. Since the computer has a complete phylogenetic record, it can assign relative taxonomic status with accuracy. Comparisons made at lower taxonomic levels, say among species within a genus, are far more effective for detecting the statistical consequences of competition — the level at which the process acts most strongly — than those that incorporate a wider taxonomic spread, say an order or a class. Here, however, the creator (GOD, or Colwell and Winkler) has a considerable advantage in knowing the likely relations between competitive intensity and the phylogenetic relationship among species, whereas such relations may be less clear to an observer in the real world. Colwell and Winkler could, if they wished, give objective and usable criteria for assigning subsets of species into "guilds" containing species with similar patterns of resource utilization, whereas such decisions about grouping real species usually involve some subjectivity.

The problems do not stop here. Sample sizes tend to be small and Type II statistical error (failure to reject an incorrect null hypothesis) tends to be high, making it very difficult to reject the null model from which the effects of competition have been excluded². Even if it can be, there are often alternative interpretations of the patterns revealed⁵. For example, habitats differ between islands and the complementarity of species distributions could

result simply from differences in habitat preferences or realized niches^{5,13}.

In the nature of things, ecologists and biogeographers can often obtain no more than circumstantial evidence. On the one hand, the studies of Colwell and Winkler illustrate some of the difficulties and pitfalls in working with such circumstantial evidence. On the other hand, by seeking to design appropriate null hypotheses that allow real patterns to be distinguished from statistical artefacts, they have achieved a constructive step in the direction indicated by Simberloff, Strong and colleagues³. Where manipulative field experiments are possible, the answers are usually much less ambiguous. Some experiments of this kind — for some taxonomic groups but not for others — point to the importance of competition in structuring natural communities^{14,15}. □

1. Harvey, P.H., Colwell, R.K., Silvertown, J.W. & May, R.M. *Ann. Rev. ecol. Syst.* **14**, 189 (1983).
2. Colwell, R.K. & Winkler, D.W. in *Ecological Communities: Conceptual Issues and the Evidence* (eds Strong, D.R., Simberloff, D., Abele, L.G. & Thistle, A.B.) 344 (Princeton University Press, Princeton, 1984).
3. Strong, D.E., Simberloff, D., Abele, L.G. & Thistle, A.B. (eds) *Ecological Communities: Conceptual Issues and the Evidence* (Princeton University Press, Princeton, 1984).
4. Raup, D.M. & Gould, S.J. *Syst. Zool.* **23**, 305 (1974).
5. Graves, G.R. & Gotelli, N.J. *Oikos* **41**, 322 (1983).
6. Grant, P.R. & Abbott, I. *Evolution* **34**, 332 (1980).
7. Case, T.J. & Siddell, R. *Evolution* **37**, 832 (1983).
8. Simberloff, D. *Am. Nat.* **112**, 713 (1978).
9. Alatalo, R. *Ecology* **63**, 881 (1982).
10. Diamond, J.M. & Gilpin, M.E. *Oecologia* **52**, 64 (1982).
11. Wright, S.J. & Biehl, C.C. *Am. Nat.* **119**, 345 (1982).
12. Connor, E.F. & Simberloff, D. *Ecol. Monogr.* **48**, 219 (1978).
13. Gilpin, M.E. & Diamond, J.M. *Oecologia* **52**, 75 (1982).
14. Schoener, T.W. *Am. Nat.* **122**, 240 (1983).
15. Strong, D.R., Lawton, J.H. & Southwood, T.R.E. *Insects on Plants: Community Patterns and Mechanisms* (Blackwell, Oxford, 1984).

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100 years ago

THE Prince of Wales, President of the International Inventions Exhibition, has fixed Monday May 4, for the opening of the Exhibition. Rapid progress is being made in all branches connected with the Exhibition. The large space set apart for machinery in motion is already being filled, whilst preparations for receiving other exhibits are well forward, some of which have arrived at the building. The Aquarium Department is receiving considerable attention, and will form a very attractive feature. Lord Onslow has lately presented 1500 exceedingly fine carp to the Aquarium, and a large number of fish indigenous to the Canadian Lakes have also been received for exhibition in the tanks. From *Nature* 31 465, 19 March 1885.

Mechanisms for homologous recombination

SIR — Fink and Petes¹ argued that the difference between a 50% frequency of crossing-over associated with meiotic gene conversion at homologous loci, on one hand, and absence of crossing-over associated with events involving repeated sequences at different locations in the genome on the other hand, suggested the existence of more than one pathway in homologous recombination. Fincham² and Whitehouse³ stressed that the frequency of crossing-over associated with meiotic gene conversion varies widely and can be much smaller than 50%. They pointed out that eukaryote recombination can occur by a single pathway that entails two steps with gene conversion occurring in the first step and reciprocal exchange in the second: variation of crossing-over association might reflect special conditions required for the second step. Nevertheless, but for different reasons than Fink and Petes, we think that recombination events occurring at homologous positions in fungi may arise via several mechanisms.

Genetic analysis in fungi shows that gene conversion can occur via distinct pathways. It strongly suggests meiotic heteroduplex DNA intermediates, involving either one of the two interacting duplexes⁴. These intermediates can be matured by mismatch correction⁵. In yeast, gene conversion can occur by the repair of double-strand gaps⁶ and it has been suggested that this might be a major pathway for meiotic recombination⁷. This diversity in the origin of gene conversion is illustrated by current models⁷⁻⁹.

The competing models for gene conversion all retain the idea of a crossed strand junction at the end of the conversion area. They predict that crossing-over arises by the resolution of the junction and therefore is associated with gene conversion. These models cannot explain simply the evidence gathered over years of genetic analysis of the crossing-over mechanism. In particular we argue that reciprocal exchanges can be spatially and temporally separated from gene conversion. In *Sordaria* cross-overs can be separated from a site of gene conversion by a region in which a marker shows normal mendelian segregation¹⁰. In yeast, mitotic gene conversion events which occur in G_1 are sometimes associated with cross-overs in G_2 ¹¹. Unified models predict that frequency of gene conversion for a central marker should be high in cells which are recombinant for the flanking markers, when the interval between the outside markers is less than the average conversion length. However, in *Ascomobolus*, reciprocal recombination selected within gene *b2* was found to be rarely associated with gene conversion in the interval¹².

Bacteriophage λ encodes functions for

two recombination systems and is a substrate for host-encoded recombination. Why should eukaryotic genetic recombination involving much larger genomes be more simple? Eukaryotes should have evolved different recombinational systems to deal with the different situations in their genomes and life histories where recombination is required.

As an alternative to continual tinkering to explain unpalatable facts, it seems worth considering schemes which invoke multiple mechanisms for recombination. We believe that the recognition of this possibility may simplify models and rejuvenate research in the whole field of recombination.

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1. Fink, G.P. & Petes, T.D. *Nature* **310**, 728 (1984).
2. Fincham, J.R.S. *Nature* **312**, 107-108 (1984).
3. Whitehouse, H.L.K. *Nature* **312**, 108 (1984).
4. Paquette, N. & Rossignol, J.-L. *Molec. gen. Genet.* **163**, 313 (1978).
5. Hastings, P.J. *et al. Curr. Genet.* **2**, 169 (1980).
6. Orr-Weaver, T.L. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 6354 (1981).
7. Szostak, J.W. *et al. Cell* **33**, 25 (1983).
8. Holliday, R. *Genet. Res.* **5**, 282 (1964).
9. Meselson, M.S. & Radding, C.M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 358 (1975).
10. Theivendirajah, K. & Whitehouse, H.L.K. *Molec. gen. Genet.* **190**, 432 (1983).
11. Roman, H. & Fabre, F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6912 (1983).
12. Rossignol, J.-L., *et al. Cold Spring Harb. Symp. quant. Biol.* (in the press).

Null hypotheses and prediction

SIR — In the past decade or so a subtle misuse of null hypotheses has become almost standard in ecology, biogeography, functional morphology and theoretical palaeontology. This comes from an overly strict adherence to the narrow Neyman-Pearson view of statistics as hypothesis-testing. The alternative approach of R.A. Fisher, himself an evolutionary biologist as well as the founder of modern statistics, emphasizes estimation, causation and the expansion of knowledge. (I simplify a complex subject.)

The difficulty here comes when a null hypothesis is placed in a privileged position, to be accepted at least provisionally until disproved. What we really want to know is how well it is supported compared with other possibilities, not all of which may be precise or even known. For this one can compare likelihoods, calculate confidence intervals, and use similar, even qualitative, approaches which treat all possibilities as reasonable *a priori*.

It may be that, because of simplicity, theoretical coherence, prior knowledge, or the like, one possibility is indeed initially pre-

ferable, or the initial likelihoods of hypotheses may be unequal. Then one can merely incorporate such knowledge via Bayes's theorem, precisely or very roughly, without the complexities of bayesian statistics. But it is rarely the case that one hypothesis is sufficiently preferable *a priori* that it should just be accepted until disproved.

For instance, the absence of adaptation for a particular structure should not be assumed until adaptation has been shown (itself often a difficult endeavour), although the possibility must be kept in mind. There are various mechanisms for nonadaptive and even inadaptive evolution at any one level^{1,2}, but most involve natural selection, and thus adaptation in some other way. Theoretical coherence based on a wide span of background knowledge actually gives a greater initial likelihood to direct or indirect adaptation without making, by itself, a precise and testable hypothesis.

Prediction is commonly associated with Popper, although it has been fashionable even in natural history since at least Cuvier's³ time, and it is commonly regarded as the only way to do science. Yet much of the evolutionary half of biology, including the basic discovery by both Darwin and Wallace, is justified deductively⁴, like mathematics. Within the domain where this is the case, hypotheses themselves are superfluous although prediction may be added, as Darwin did, to give additional justification.

We must not let the simplicity of a cookbook methodology blind us to real diversity. As Harvey *et al.*⁵ recently quoted in another context, from Tukey⁶, "Far better an approximate answer to the *right* question, which is often vague, than an exact answer to the *wrong* question, which can always be made precise."

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1. Van Valen, L.M. *Amer. Nat.* **94**, 305-308 (1960).
2. Gould, S.J. & Lewontin, R.C. *Proc. R. Soc. Lond B* **205**, 581-598 (1979).
3. Rudwick, M.J.S. *The Meaning of Fossils* (Macdonald, London, 1972).
4. Van Valen, L.M. in *Conceptual Issues in Ecology* (ed. Saarela, E.) 323-343. (Reidel, Dordrecht, 1982).
5. Harvey, P.H., Colwell, R.K., Silvertown, J.W. & May, R.M. *A. Rev. Ecol. Syst.* **14**, 189-211 (1983).
6. Tukey, J.W. *Annals. math. Stat.* **33**, 1-67 (1962).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

Study of magnetism in osmium cluster compounds as molecular models for small metallic particles

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The magnetic susceptibilities of a series of cluster carbonyl compounds of osmium have been measured by a high-sensitivity Faraday method and the evolution of certain aspects of 'metallic' behaviour in the high-nuclearity clusters is revealed by a steady increase in the excess molecular susceptibility as the cluster size increases. We also consider here 'metallic' status in the cluster compounds of other transition elements.

THE physical and chemical properties of small metallic particles with diameters $< 100 \text{ \AA}$ (refs 1–6) are interesting because of their importance in heterogeneous catalysis and their position as intermediaries between the atomic and (bulk) metallic regimes. It is often asked in what manner, and at what nuclearity, atomic and molecular properties admit 'metallic' behaviour^{1,6–13}. This description involves, to first order, the neglect of inter-cluster interactions and deals primarily with the evolution of the electronic structure of isolated cluster states. Representative examples include ultra-fine dispersed metal catalysts^{2–4}, 'naked' metal clusters formed via the controlled aggregation of metal atoms in low-temperature matrices^{14–16}, and, the subject of the present investigation, inorganic cluster compounds of the transition elements¹⁷. The study of small metallic particles poses severe experimental problems because of unavoidable distributions of particle size within a sample and the necessity for high-vacuum conditions to avoid contamination with oxide species¹. In contrast, carbonyl cluster compounds of, for example, Os, Rh and Pt, of generic formula $M_m(\text{CO})_n^Q$, with $Q \geq 0$, $n > m$, are well characterized molecular species, obtainable in samples of very high purity, and many are air-stable. The cluster carbonyls of these three elements have been shown by X-ray crystallography to exhibit many different geometries (Fig. 1), which are fragments of extended metallic lattices (hexagonal close-packed (h.c.p.); face-centred cubic (f.c.c.); body-centred cubic (b.c.c.)), or resemble other types of close packing (for example, polytetrahedral, icosahedral), theoretically favourable for particles containing a few dozen atoms^{17–20}.

The compounds in Fig. 1 represent metallic cluster fragments isolated from adjacent cluster units by inert sheaths of carbonyl ligands; these cluster units are fundamentally different from, for example, those within the ternary molybdenum chalcogenides (ref. 21 and references therein), often called Chevrel phases, $M_x\text{Mo}_6\text{X}_8$ (where M represents a rare earth or other metallic element and X is S, Se or Te), in which the chalcogen atoms occupy bridging positions between the metal cluster units, and extensive inter-cluster interactions give rise to itinerant electron behaviour at ambient temperatures, and superconductivity below $\sim 15 \text{ K}$.

The transition-metal cluster carbonyls, therefore, may be ideal systems for monitoring the genesis of the electronic structure of particulate transition metals. We report here a systematic study of magnetism in the cluster carbonyls of osmium.

Magnetic measurements

The magnetic susceptibilities of the various Os cluster compounds were measured over the range 1.5–300 K using a high-sensitivity computer-interfaced Faraday balance, described elsewhere²². Samples were suspended on a 1-mm quartz rod from the arm of a Cahn RG electrobalance. The sample container ($4.2 \times 10 \text{ mm}$) was fashioned from high-purity Spectrosil quartz. An electromagnet (Eastern Scientific Instruments) supplied variable fields up to 11.8 kG. The magnetic field gradient was measured using $\text{HgCo}(\text{SCN})_4$ as a standard and varied by $< 2\%$. Temperature was controlled ($\pm 0.1 \text{ K}$) and monitored with a digital temperature controller (Oxford Instruments, DTC 2) using a Au/Fe versus chromel/P thermocouple. A calibrated silicon diode resistance thermometer was used to measure the sample temperature, independently of the controlling thermocouple, over the entire temperature range. The Cahn balance was operated on the 1-g scale, on which a sensitivity in ideal conditions of 0.0005 mg can be achieved; in normal operating conditions, the sensitivity was only $\sim 0.001 \text{ mg}$ and the reproducibility was $\sim 0.001 \text{ mg}$. A typical error estimate in the measurement of magnetic susceptibility is $\pm 10 \times 10^{-6} \text{ e.m.u. mol}^{-1}$ for $\text{Os}_3(\text{CO})_{12}$ and $\pm 30 \times 10^{-6} \text{ e.m.u. mol}^{-1}$ for the cluster $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$. For each sample, magnetic measurements were taken at three values of the magnetic field, at 8.8, 10.5 and 11.8 kG. The total measured susceptibility values of the Os cluster compounds at room temperature, which are (overall) diamagnetic, range from $-170(\pm 10) \times 10^{-6} \text{ e.m.u. mol}^{-1}$ for $\text{Os}_3(\text{CO})_{12}$ to $-700(\pm 40) \times 10^{-6} \text{ e.m.u. mol}^{-1}$ for $\text{Os}_{10}\text{C}(\text{CO})_{24}\text{I}_2$ and have been corrected for the intrinsic magnetism of the sample container.

The experimental quantity of most direct interest is the excess molecular susceptibility, χ_{ems} , of the Os cluster compounds, which represents the difference between the magnetic susceptibility of the molecular complex (Fig. 1) and the sum of those species from which the complex is composed. The natural choice for these constituents is Os and H atoms, freely rotating CO molecules and, in the case of $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$, an additional C atom (the interstitial C atom, Fig. 1). Reliable values for Os ($-66 \times 10^{-6} \text{ e.m.u. mol}^{-1}$), I ($-52 \times 10^{-6} \text{ e.m.u. mol}^{-1}$), C ($+5.0 \times 10^{-6} \text{ e.m.u. mol}^{-1}$) and H ($-2.93 \times 10^{-6} \text{ e.m.u. mol}^{-1}$) are given by König²³. The measured magnetic susceptibility for gaseous CO is $-9.8 \times 10^{-6} \text{ e.m.u. mol}^{-1}$. The observed molar susceptibilities and derived excess molecular susceptibilities at 298 K and 11.8 kG for six cluster compounds of Os are given in Table 1 and these limiting high-temperature values are plotted as a function of cluster nuclearity in Fig. 2. Note that the

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experimental excess molecular susceptibilities (χ_{ems}) are considerably larger than the intrinsic errors in our measurements. (For example, for the low-temperature susceptibility of $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$ to arise from Os (IV) impurities, some 20% of the molecules in the sample would have to be Os(IV) complexes. No evidence of such complexes was found by infrared spectroscopy or X-ray diffraction.)

The magnetic susceptibilities of the entire series of Os clusters are truly temperature-independent down to 1.5 K, except for the decanuclear hydrido-cluster, $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$, which shows a temperature-dependent paramagnetism at low temperature, approximating closely to a Curie-type behaviour.

Discussion

The excess molecular susceptibilities (Fig. 2), χ_{ems} , are most naturally interpreted as arising from a Van Vleck paramagnetic contribution to the susceptibility. Somewhat related magnetic behaviour has been observed previously in transition metal (molecular) complexes that have nominal singlet ground states, for example, in $\text{Cr}_2\text{O}_7^{2-}$, MnO_4^- and WO_4^{2-} complexes, as well as in Co (III) complexes possessing a low-lying excited state²⁴⁻²⁶. However, of particular importance here is the marked increase in χ_{ems} with increasing cluster size (Fig. 2) and how this relates to our view of the electronic structure of these materials.

In the present instance, a Van Vleck contribution²⁶ to the observed susceptibility will arise from modification of the cluster wavefunction by the external magnetic field. The modified wavefunction is described by the admixture of excited states in which electrons are promoted from delocalized bonding orbitals of the cluster to the delocalized antibonding orbitals. As the second-order perturbation expression for this paramagnetic contribution contains terms inversely proportional to the excitation energies between the occupied and unoccupied molecular orbitals^{26,27}, the increase in χ_{ems} with increasing cluster size suggests that the excitation energy gap decreases correspondingly. This interpretation (discussed below) would be invalidated if the matrix element $\langle n|\mu_z|0\rangle$, connecting the ground state $|0\rangle$ with the excited state $|n\rangle$ of energy $(E_n - E_0)$ above the ground state, changed significantly with cluster size. This, however, is improbable.

That relativistic effects, of which spin-orbit coupling is the best known, may well be significant in a heavy atom such as Os does not invalidate our qualitative interpretation of χ_{ems} . Although a closed-shell ground state will contain admixtures of states having non-zero total-spin, S , in relativistic theory, such a closed relativistic configuration will still be symmetric under time-reversal; the operator describing the interaction of the closed shell with the external magnetic field is antisymmetric and thus, under this operation, the first-order energy of interaction vanishes. Hence, the observed magnetic contribution consists, apart from the usual diamagnetic term, of second-order effects in which the wavefunction is modified by the magnetic field. Note that attempts to account for the delocalization of cluster bonding electrons in molecular orbitals would lead to larger diamagnetic terms, as the electrons would necessarily have larger effective radii than those confined to single Os atoms; and the values of the excess molecular susceptibility would then be increased with respect to those given in Table 1.

Several limiting descriptions are applicable to this Van Vleck temperature-independent paramagnetism (TIP), sometimes called the 'high-frequency' or 'second-order Zeeman' paramagnetism (see ref. 27 for a detailed review of the four important situations). Note that the same energy denominator that governs the magnitude of the Van Vleck paramagnetism is also responsible for the important paramagnetic contribution to the chemical shielding constant in nuclear magnetic resonance (NMR)²⁸. However, one of the matrix elements in the NMR situation differs slightly from that in the Van Vleck paramagnetism.

In the normal case where the characteristic energy gap $(E_n - E_0)$, separating occupied from vacant orbitals, is large compared with the thermal energy, that is, $(E_n - E_0) \gg kT$, the induced paramagnetism is truly temperature independent^{27,29}. The Os clusters would, at first sight, be expected to have a pure spin-singlet magnetic ground state. Thus, theories of bonding have successfully concentrated on the number of skeletal electron pairs required to bind the metal cluster unit, implicitly assuming a pure diamagnetic ground state^{30,31}. The magnetic behaviour (Fig. 2) suggests that this picture is reasonable for small clusters, but becomes inappropriate for larger clusters in which there is obviously a large reduction in the frontier-orbital energy gap. Overall, then, we attribute the increasing Van Vleck TIP contribution for the high-nuclearity clusters primarily to a reduction in $E_n - E_0$, identified here as a measure of the separation of the frontier orbitals in the cluster³². A theoretical molecular

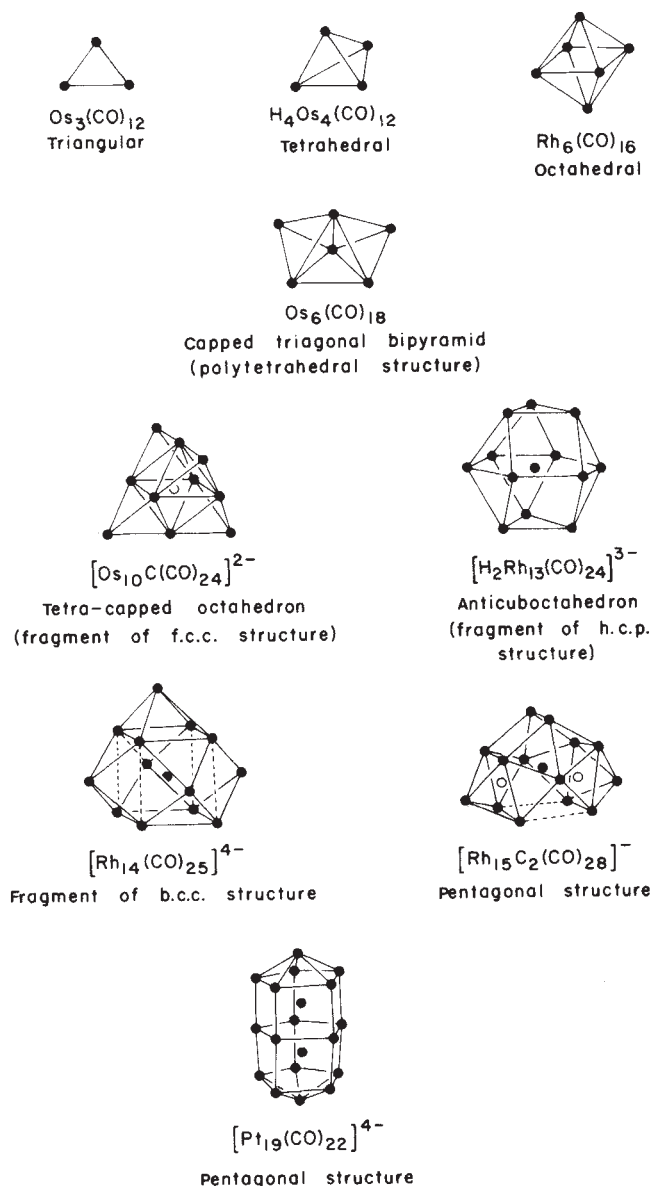


Fig. 1 A representation of typical metal atom geometries in transition metal carbonyls. For clarity, the carbonyl ligands have been omitted. Open circles denote interstitial carbon atoms in the cluster compound. **Materials.** The compounds $\text{Os}_3(\text{CO})_{12}$, $\text{H}_2\text{Os}_3(\text{CO})_{10}$, $\text{H}_4\text{Os}_4(\text{CO})_{12}$, $\text{Os}_7(\text{CO})_{21}$, $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$ and $\text{Os}_{10}\text{C}(\text{CO})_{24}\text{I}_2$ were prepared by existing methods^{18,46}. The purity and composition of the samples were carefully checked by TLC, infrared spectroscopy and, in the case of the clusters containing < 10 Os atoms, by mass spectrometry (AEI MS12, 100–150 °C). The elemental purity of all compounds was also tested by atomic absorption spectroscopy; the elements Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb and Pd were tested in a 47-mg sample of the decanuclear cluster $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$ and were undetectable down to the limits of detection of the absorption spectrometer, which were 1 p.p.m. for Fe and Zn, and 0.1 p.p.m. for the remaining elements.

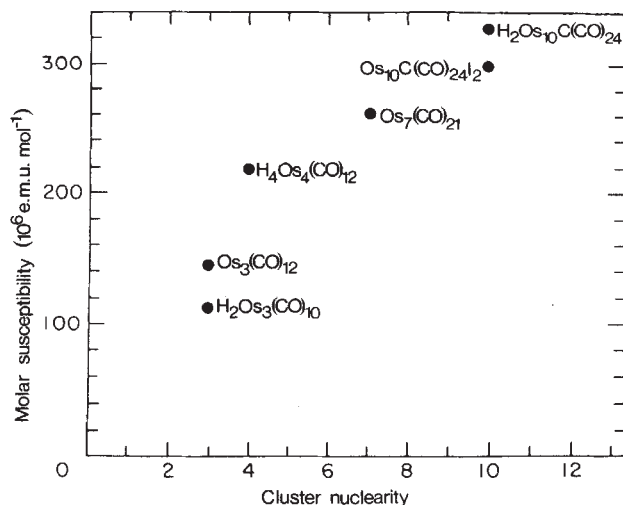


Fig. 2 Magnetism in Os cluster carbonyls. The variation of the high-temperature (298 K) excess molecular susceptibility, χ_{ems} , as a function of cluster nuclearity.

orbital study has been made³³ on $\text{Ru}_3(\text{CO})_{12}$, isostructural with $\text{Os}_3(\text{CO})_{12}$, to aid interpretation of its ultraviolet/visible electronic spectrum³⁴. The first excited state in this D_{3h} molecule lies ~ 3 eV ($24,000 \text{ cm}^{-1}$) above the ground state. Although optical transitions to this particular state are forbidden, it is of the correct symmetry to give a non-zero value for $\langle n|\mu_z|0\rangle^2$. This leads to a value for the TIP susceptibility of $\sim 180 \times 10^{-6} \text{ e.m.u. mol}^{-1}$, if we estimate $\langle n|\mu_z|0\rangle^2$ from symmetry arguments³⁵, which is indeed consistent with our observed value for $\text{Os}_3(\text{CO})_{12}$. The next three excited states correspond to allowed optical transitions, but are magnetically forbidden, so that the sum over excited states in the calculation of the excess molecular paramagnetism is dominated by the HOMO-LUMO interaction, in this case.

It is important to note that these cluster molecules are tightly bound and contain electronic energy levels with values of $(E_n - E_0)$ greatly different from those of the individual interacting metal centres³⁶. If the cluster units were loosely bound, as in the case of van der Waals-type clusters of noble gas atoms^{19,37}, their magnetic properties would simply be those of an assembly of mononuclear metal carbonyl fragments. This is clearly not the case for the title systems.

The ultraviolet/visible spectra of larger clusters show a trend towards absorption at lower frequencies. Chini³² has noted that this trend can be taken to reflect the separation of frontier orbitals, which clearly correlates with the colour sequences found in the Os clusters and carbonylmetallates. If excited states of suitable symmetry are present, this would give rise to an increased TIP susceptibility as the cluster size increases (Fig. 2). Unfortunately, a more detailed discussion would be premature, as our knowledge of the excited electronic states in cluster molecules is extremely limited²⁰. Very few data are available for the electronic absorption spectra of high-nuclearity clusters. Satisfactory quantitative theoretical calculations on large many-electron clusters of metal atoms with large spin-orbit coupling constants cannot at present be performed. Even the relatively simple case of the dichromium molecule, Cr_2 , has given rise to considerable disagreement among the results from different methods of calculation³⁸. Extended Hückel molecular-orbital calculations using Rh atom parameters have been partially successful, but an incorrect number of cluster valence molecular orbitals was obtained³⁹ for (ligand-free) 10-atom clusters having the geometry of $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$.

If one traces the evolution of the Van Vleck paramagnetic contribution from the situation when $(E_n - E_0) \gg kT$, to $(E_n - E_0) \ll kT$, then the second-order Zeeman effect transforms smoothly from temperature-independent paramagnetism, to a Curie-type paramagnetism^{7,29}. For the situation in which the

Table 1 Magnetic susceptibility data for Os clusters (298 K)*

Cluster	$\chi_m(\text{obs})$ ($10^6 \text{ e.m.u. mol}^{-1}$)	χ_{ems} ($10^6 \text{ e.m.u. mol}^{-1}$)
$\text{Os}_3(\text{CO})_{12}$	-170 ± 10	$+146 \pm 10$
$\text{H}_2\text{Os}_3(\text{CO})_{10}$	-188 ± 10	$+114 \pm 10$
$\text{H}_4\text{Os}_4(\text{CO})_{12}$	-200 ± 10	$+219 \pm 10$
$\text{Os}_7(\text{CO})_{21}$	-405 ± 20	$+263 \pm 20$
$\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$	$-580 \pm 30^\dagger$	$+326 \pm 30$
$\text{Os}_{10}\text{C}(\text{CO})_{24}\text{I}_2$	-700 ± 40	$+304 \pm 40$

* All values are corrected for the susceptibility of the Spectrosil container. The experimental data are temperature-independent down to 1.5 K, except for $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}^\dagger$, which is temperature-dependent below ~ 150 K.

characteristic energy gap is sufficiently small to be comparable with kT , namely, $(E_n - E_0) \ll kT$, the resulting contribution to the magnetization gives a susceptibility contribution proportional to $\langle n|\mu_z|0\rangle^2/kT$. This has the usual Curie form, although here the mechanism of magnetization is by the admixture of virtual excited states into the ground state, whereas for 'free' (Curie) spins, the mechanism is that of redistribution among the spin states²⁹. Note that, in this case, the energy parameter $(E_n - E_0)$ does not enter into the paramagnetic contribution. In the intermediate regime, Figgis²⁷ has noted that the observed magnetic susceptibility contains contributions from both types of effect.

We have reported⁴⁰ that the decanuclear cluster $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$ behaves in this fashion, revealing a strong temperature-dependent paramagnetism at low temperatures. In addition, preliminary magnetic measurements on several high-purity anionic decanuclear Os clusters also show a Curie-type paramagnetism in the magnetic susceptibility. In terms of the Van Vleck theory of magnetism, this strong paramagnetic susceptibility is nominally symptomatic of a diamagnetic state in which the energy gap separating the ground state and excited state for mixing is comparable with kT . However, we caution against accepting this statement as a rigorous characterization of $(E_n - E_0)$ from the susceptibility data, as the thermodynamic and magnetic properties depend on the overall pattern of energy levels⁴¹⁻⁴³. In particular, it is not clear whether the assumption of Boltzmann-type statistics is indeed valid for a metal cluster of this size, that is, whether Fermi-Dirac statistics would not be more appropriate in this particulate metal regime.

An alternative description, much discussed in solid-state physics^{1,11,12}, approaches the problem from the bulk metallic regime and considers the consequences of the fragmentation of a metallic sample into a 'particulate' metal. Briefly, the finite extension of the electron wavefunction within a particulate metal causes a changeover of the electron levels from quasi-continuous to discrete. The results of theoretical calculations of the magnetic properties of particulate metals depend on the assumptions made concerning the overall distribution of energy levels, for example, whether they are equally spaced, or in a Poisson distribution, and whether or not degeneracy of levels is allowed^{41,42}. The unitary ensemble calculation, most appropriate for metals (such as Os) with high spin-orbit coupling, is the least studied, but a transition from the temperature-independent Pauli paramagnetism of bulk metals to a Curie-type behaviour in the magnetic susceptibility has been predicted for both odd- and even-electron metals^{12,41,42}. Experimental observations of enhanced low-temperature susceptibility have been made for particles of metals as diverse as lithium⁴⁴ (odd electron), magnesium⁴⁵ and platinum⁴² (even electron).

In terms of its magnetic properties, the decanuclear cluster $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$ may be representative of a small metallic particle in the so-called quantum-size effect regime.

We note that the structure of $\text{Os}_{10}\text{C}(\text{CO})_{24}\text{I}_2$ is considerably more 'open' than the hydrido-cluster $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$, because of the bonding contribution of the iodine atoms⁴⁶. It may be more correct in this context to regard the iodo-cluster as an octanuclear cluster with two extra peripheral (edge bridging)

Os atoms. In $\text{Os}_{10}\text{C}(\text{CO})_{24}\text{I}_2$, only temperature-independent paramagnetism is observed (Fig. 2).

The low-temperature paramagnetism of $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$ has been further investigated by electron paramagnetic resonance (EPR). Spectra of polycrystalline $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$ showed a broad almost-symmetrical lorentzian resonance centred at $g = 2.27 \pm 0.02$. As the temperature was raised, the peak-to-peak linewidth, ΔH_{pp} , increased from ~ 50 G at 18 K to ~ 80 G at 77 K.

EPR signals with characteristic dysonian (asymmetrical) lineshapes are given by conduction electrons in bulk metals⁴⁸. The mechanism of electron spin relaxation in metals has been discussed in detail by Elliott^{49,50}, who showed that spin relaxation occurs through the combined agencies of electron-phonon scattering and spin-orbit coupling. For heavy bulk metals in which spin-orbit interactions are large, for example, Os with a spin-orbit coupling constant of $\sim 1,000 \text{ cm}^{-1}$, electron spin relaxation times are expected to be extremely short and EPR resonances are anticipated to be broadened beyond detection at ambient temperatures⁵¹. Using values of the resistivity relaxation rate (τ_R^{-1}), derived from measurements on bulk Os metal, and taking $g = 2.27$, gives an electron spin lattice relaxation rate of $2 \times 10^{14} \text{ s}^{-1}$; the corresponding EPR linewidth would be $\sim 1 \times 10^7$ G, clearly undetectable. But a relaxation rate of $\sim 1 \times 10^9 \text{ s}^{-1}$ is indicated from the EPR of $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$.

This large reduction in electron spin relaxation rate from the bulk Os value is also consistent with a description of $\text{H}_2\text{Os}_{10}\text{C}(\text{CO})_{24}$ as a particulate metal. It is well established that electron spin relaxation processes are inhibited or suppressed in this quantum-size effect regime^{12,52}. Briefly, in a particulate metal the electronic energy level spacing may become comparable with the magnetic Zeeman energy. Quenching of the electron spin relaxation processes in small metallic particles arises because electron (spin) transitions between these discrete energy levels⁵² are restricted as opposed to the situation in a bulk metallic sample⁴⁹. Therefore, this quantum-size effect considerably narrows the EPR absorption and it may become detectable. Moreover, if the particle diameter is smaller than the characteristic microwave skin depth, the lineshape changes from dysonian to lorentzian^{48,50}. EPR signals of lorentzian shape have indeed been observed for small particles of metals with both odd (for example, alkalis⁵⁰, silver⁵³⁻⁵⁵) and even (for example, magnesium⁴⁴ and platinum⁵⁶) numbers of valence electrons. Similarly, small metallic particles studied⁵⁷ by EPR in alkali halide (host) crystals include the (even electron) 2nd row transition metals Pd and Cd. There have been no reports of Os colloids isolated in these host materials.

Conclusions

This study of the magnetic properties of a series of Os clusters demonstrates experimentally the manner in which the changeover from molecular to 'particulate-metal' behaviour may develop as a cluster aggregate forms. The temperature-independent Van Vleck paramagnetism increases sequentially with cluster size as the molecular electronic states begin to develop into the band or continuum regime and ultimately terminates in the characteristic Pauli paramagnetism in the bulk metallic state. Moreover, electronic states within the particulate-metal regime, in which quantum-size effects may be operative, can exhibit enhanced paramagnetism compared with both their localized- and itinerant-electron counterparts. Similar observations have been made on particulate samples of several metals. There are other theoretical predictions and experimental observations of enhanced paramagnetism in this 'narrow-band' regime separating the metallic and insulating states^{58,59}.

We have demonstrated the considerable sensitivity of magnetic measurements in detecting the onset of certain aspects of what may be termed 'metallic' behaviour. This has an important bearing on the question⁶, 'how many atoms are needed in an aggregate or cluster to mimic effectively the bulk properties of a metal?' The answer must obviously depend on which particular property of the metal one wishes to describe. Transport properties (for example, conductivity, Hall effect) require, as a prere-

quisite for detection of metallic character, the complete ionization of an electron from its parent site, which typically involves energy changes in the range 10^{-1} – 10 eV. As far as the magnetic properties are concerned, modifications to the intra- and inter-unit exchange energy in the metal cluster as the nuclearity increases need only be of the order of magnitude of the magnetic Zeeman or hyperfine energies (both typically in the range 10^{-4} eV) for noticeable changes to be observed. These types of experimental considerations are fundamental to any consideration of 'metallic' characteristics. Several authors^{1,7-10,60} have suggested a criterion for bulk properties in clusters based on the width of the electron d band, as determined experimentally, for example, by photoelectron spectroscopy. Such a criterion obviously involves large energy terms in the range 1–10 eV. Similarly, quantum mechanical calculations aimed at reproducing bulk-metal d-band widths in clusters seem certain to require several hundreds, or even thousands, of atoms before metallic status would be conferred on the aggregate⁷⁻¹⁰. These approaches may overlook and obscure many interesting magnetic and optical properties of clusters in the particulate-metal regime.

We have suggested that transition metal carbonyl cluster compounds represent ideal model systems for monitoring the genesis of a particulate metal from individual molecular units. Transition metal cluster compounds of slightly higher nuclearity than those examined here may show very interesting electronic properties, possibly arising from a polarization catastrophe⁶ within the cluster units themselves.

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1. Borel, J.-P. & Buttet, J. (eds) *Small Particles and Inorganic Clusters*, Surf. Sci. **106**, (1981).
2. Davis, S. C. & Klabunde, K. J. *Chem. Rev.* **82**, 153–208 (1982).
3. Maugh, T. H. *Science* **219**, 474–477 (1983).
4. Bourdon, J. (ed.) *Growth and Properties of Metal Clusters*, Proc. 32nd Int. Meet. Soc. Chim. Phys. Villeurbanne (Elsevier, Amsterdam, 1980).
5. Muetterichs, E. L., Rhodin, T. N., Band, E., Bruker, C. F. & Pretzer, W. R. *Chem. Rev.* **79**, 91–137 (1979).
6. Edwards, P. P. & Sienko, M. J. *Int. Rev. Phys. Chem.* **3**, 83–137 (1983).
7. Baetzold, R. C. *Inorg. Chem.* **20**, 118–123 (1981).
8. Bachmann, C., Demuyneck, J. & Veillard, A. *Faraday Symp. chem. Soc.* **14**, 170–179 (1980).
9. Demuyneck, J., Rohmer, M.-M., Strich, A. & Veillard, A. *J. chem. Phys.* **75**, 3443–3453 (1981).
10. Yang, C. Y., Johnson, K. H., Salahub, D. R., Kaspar, J. & Messmer, R. P. *Phys. Rev. B* **24**, 5673–5692 (1981).
11. Fröhlich, H. *Physica* **4**, 406–412 (1937).
12. Kubo, R. *J. Phys. Soc. Japan* **17**, 975–986 (1962); *J. Phys., Fr.* **38**, Suppl. 7, C2-69 (1977).
13. Abeles, B., Sheng, Ping, Coutts, M. D. & Arie, Y. *Adv. Phys.* **24**, 407–461 (1975).
14. Ozin, G. A. *Faraday Symp. chem. Soc.* **14**, 7–64 (1980).
15. Howard, J., Sutcliffe, R., Tse, J. S. & Mile, B. *Chem. Phys. Lett.* **94**, 561–564 (1983).
16. Thompson, G. A., Tischler, F. & Lindsay, D. M. *J. chem. Phys.* **78**, 5946–5953 (1983).
17. Johnson, B. F. G. (ed.) *Transition Metal Clusters* (Wiley, Chichester, 1980).
18. Johnson, B. F. G. & Lewis, J. *Adv. inorg. Chem. Radiochem.* **24**, 225–355 (1981).
19. Hoare, M. R. & Pal, P. *Nature Phys. Sci.* **236**, 35–37 (1972); *Adv. Phys.* **24**, 645–678 (1975).
20. Woolley, R. G. *Nouv. J. Chim.* **5**, 441–446 (1981); in *Transition Metal Clusters* (ed. Johnson, B. F. G.) 607–659 (Wiley, Chichester, 1980).
21. Yvon, K. *Curr. Topics Mater. Sci.* **3**, 53–129 (1979).
22. Johnson, D. C. thesis, Cornell Univ. (1983).
23. König, E. in *Landolt-Börnstein, New Ser., Group II Atomic and Molecular Physics* Vol. 2 (ed. Hellwege, K.-H.) 1 (Springer, Berlin, 1966).
24. Freed, S. & Kasper, C. J. *Am. chem. Soc.* **52**, 4671–4679 (1930).
25. Carlin, R. H. & van Duijneveldt, A. J. *Magnetic Properties of Transition Metal Compounds* (Springer, New York, 1977).
26. Van Vleck, J. H. *Electric and Magnetic Susceptibilities* (Oxford University Press, 1932).
27. Figgis, B. N. *Introduction to Ligand Fields*, 252–256 (Interscience, New York, 1966).
28. Freeman, R., Murray, G. R. & Richards, R. E. *Proc. R. Soc. A* **242**, 455–466 (1957).
29. Kittel, C. *Introduction to Solid State Physics* 2nd edn 578–579 (Wiley, New York, 1963).
30. Wade, K. in *Transition Metal Clusters* (ed. Johnson, B. F. G.) Ch. 3 (Wiley, Chichester, 1980).
31. Mingos, D. M. P. *Nature Phys. Sci.* **236**, 99–102 (1972).
32. Chini, P. *Pure appl. Chem.* **23**, 489–503 (1970).
33. Delley, B., Manning, M. C., Ellis, D. E., Berkowitz, J. & Trogler, W. C. *Inorg. Chem.* **21**, 2247–2253 (1982).
34. Tyler, D. R., Levenson, R. A. & Gray, H. B. *J. Am. chem. Soc.* **100**, 7888–7893 (1978).
35. Ballhausen, C. J. *Introduction to Ligand Field Theory*, 147 (McGraw-Hill, New York, 1962).
36. Hoffmann, R., Schilling, B. E. R., Bau, R., Kausz, H. & Mingos, D. M. P. *J. Am. chem. Soc.* **100**, 6088–6093 (1978).
37. Jortner, J. *Ber. Bunsenges. Phys. Chem.* **88**, 188–201 (1984).
38. Michalopoulos, D. L., Geusic, M. E., Hansen, S. G., Powers, D. E. & Smalley, R. E. *J. phys. Chem.* **86**, 3914–3916 (1982).
39. Ciani, G. & Sironi, A. *J. organomet. Chem.* **197**, 233–248 (1980).

40. Benfield, R. E., Edwards, P. P. & Stacy, A. M. *JCS chem. Commun.*, 525-526 (1982).
41. Denton, R., Mühlischlegel, B. & Scalapino, D. J. *Phys. Rev. Lett.* **26**, 707-711 (1971); *Phys. Rev. B* **7**, 3589-3607 (1973).
42. Marzke, R. F. *Catal. Rev. Sci. Engng* **19**, 43-65 (1979).
43. Knight, W. D. J. *Vacuum Sci. Technol.* **10**, 705-712 (1973).
44. Borel, J.-P. & Millet, J.-L. *J. Phys., Fr.* **38**, 115-119 (1977).
45. Millet, J.-L. & Borel, J.-P. *Surface Sci.* **106**, 403-407 (1981).
46. Lewis, J. & Johnson, B. F. G. *Pure appl. Chem.* **54**, 97-112 (1982).
47. Dyson, F. J. *Phys. Rev.* **98**, 349-359 (1955).
48. Feher, G. & Kip, A. F. *Phys. Rev.* **98**, 337-348 (1955).
49. Elliott, R. J. *Phys. Rev.* **96**, 266-279 (1954).
50. Edmonds, R. N., Guy, S. C., Edwards, P. P. & Johnson, D. C. *J. phys. Chem.* (in the press); Edmonds, R. N. & Edwards, P. P. *Proc R. Soc.* (in the press).
51. Monod, P. & Beuneu, F. *Phys. Rev.* **B18**, 2422-2425 (1978); **19**, 911-916 (1979).
52. Kawabata, A. *J. phys. Soc. Japan* **29**, 902-911 (1970).
53. Millet, J.-L. & Borel, J.-P. *Solid St. Commun.* **43**, 217-220 (1982).
54. Hermerschmidt, D. & Haul, R. *Ber. Bunsenges. phys. Chem.* **84**, 902-907 (1980).
55. Ozin, G. A. *J. Am. chem. Soc.* **102**, 3301-3303 (1980).
56. Gordon, D. A., Marzke, R. F. & Glaunsinger, W. S. *J. Phys., Fr.* **38**, C2-87-91 (1977).
57. Hughes, A. E. & Jain, S. C. *Adv. Phys.* **28**, 717-828 (1979).
58. Mott, N. F. *Metal-Insulator Transitions* (Taylor & Francis, London, 1974).
59. Holcomb, D. F. in *The Metal-Non-Metal Transition in Disordered Systems* (eds Fredman, L. P. & Tunstall, D. P.) 251-284 (19th Scottish Universities Summer School in Physics, Edinburgh, 1978).
60. Hamilton, J. F., Apai, G., Lee, S. T. & Mason, M. G. in *Growth and Properties of Metal Clusters* (ed. Bourdon, J.) 387-391 (Elsevier, Amsterdam, 1980).

Hydrogen bonding and biological specificity analysed by protein engineering

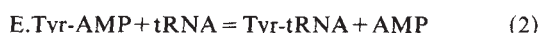
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The role of complementary hydrogen bonding as a determinant of biological specificity has been examined by protein engineering of the tyrosyl-tRNA synthetase. Deletion of a side chain between enzyme and substrate to leave an unpaired, uncharged hydrogen-bond donor or acceptor weakens binding energy by only 0.5–1.5 kcal mol⁻¹. But the presence of an unpaired and charged donor or acceptor weakens binding by a further ~3 kcal mol⁻¹.

THE hydrogen bond is a ubiquitous feature of biological interactions: it is essential in determining the structure of proteins and nucleic acids; it is a major determinant of specificity in enzyme catalysis and in biological information transfer; and it can influence directly the rate of enzymatic reactions by stabilizing the ionic charges formed in the transition state. Hydrogen bonding in macromolecules and their complexes in aqueous solution is a complex phenomenon because water competes for the hydrogen-bonding sites^{1,2}. The calculation of the overall energetics is consequently difficult and there is little knowledge of the energies involved³. Here we apply an experimental approach to the problem, using site-directed mutagenesis to produce mutant enzymes that differ in their abilities to form hydrogen bonds with their substrates. The interaction energies can be calculated from kinetic data on the modified enzymes.

Our experimental system is the tyrosyl-transfer RNA synthetase from *Bacillus stearothermophilus*. This enzyme catalyses the aminoacylation of tRNA^{Tyr} in a two-step reaction; activation of the amino acid to form the enzyme-bound tyrosyl adenylate complex followed by transfer to tRNA^{Tyr} (ref. 4).



The particular suitability of this enzyme (E) for obtaining accurate kinetic data and its other favourable characteristics for systematic site-directed mutagenesis have been described in detail elsewhere⁵. The three-dimensional structure of the enzyme and its bound aminoacyl adenylate are known from X-ray crystallography⁶⁻⁹. Eleven possible hydrogen bonds have been identified that may be formed between the enzyme and substrate, eight of which are from amino-acid side chains that may be mutated (Fig. 1). We are systematically mutating these residues to measure the energetics of their interactions and to test whether hydrogen bonds are actually involved¹⁰. In all cases, the mutations lead to a smaller side chain that either lacks or has a modified hydrogen-bond donor or acceptor. Our initial data are consistent with hydrogen bonding being an exchange reaction whereby the hydrogen-bond donors and acceptors on the free enzyme and free substrate break their bonds with water on forming the bonds in the enzyme-substrate complex^{5,11,12}. Here we analyse the effects of mutation of several different types of

hydrogen bonds, including bonds from residues involved in determining the high specificity of the enzyme for tyrosine rather than phenylalanine (Tyr 34), bonds for simple binding of the substrate (Cys 35, His 48, Thr 51, Tyr 169) and a bond closer to the seat of reaction (Gln 195). These bonds may be classified further according to charge; most are between uncharged polar residues, but some involve a charged group on either the enzyme or substrate.

Experimental observations

Our experimental results for the activation of tyrosine are listed in Table 1. The contributions of the side chains to binding were calculated from the values of k_{cat}/K_M as described previously (ref. 5, Table 2). Note that the data refer to the binding of the substrate in the transition state. Such data give, in general, the most reliable measurements of incremental binding energies as the effects of strain, nonproductive binding and induced fit do not affect k_{cat}/K_M (ref. 13). The data for the activation of phenylalanine by the wild-type enzyme and a mutant in the specificity pocket are listed in Table 3. The contributions of side chains to binding energy, discussed in more detail below, may be summarized and classified thus: (1) deletion of a side chain on the enzyme that forms a good hydrogen bond with an uncharged group on the substrate weakens binding energy by only 0.5–1.5 kcal mol⁻¹; (2) deletion of an uncharged side chain on the enzyme that forms a hydrogen bond with a charged group on the substrate weakens binding by ~3.5–4.5 kcal mol⁻¹—only two relevant experiments were performed for this category and so the range could be much wider, but the crucial point is that these energies are considerably higher than in (1); (3) deletion of a group that forms a too-long hydrogen bond actually improves binding.

Effects of removal of side chains

Our measurements on the mutated enzymes give the overall contributions of the side chains to binding. The hydrogen bond donors/acceptors that are removed on mutagenesis are the only portions of the side chains in direct contact with the substrate. But the mutation of an amino acid in a protein from a larger side chain to a smaller may have further structural consequences superimposed on the energetics of the hydrogen bonding. Any fine structure analysis must consider these possibilities and

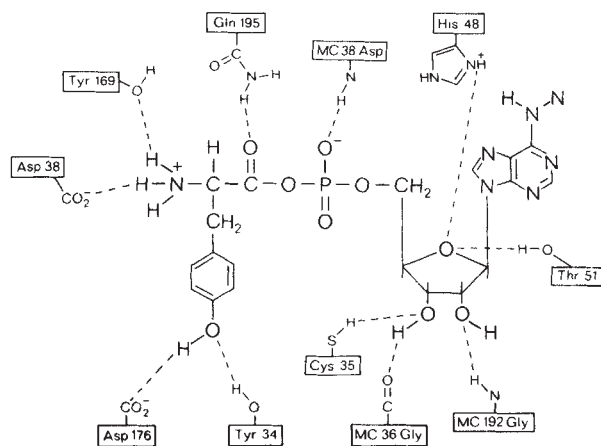


Fig. 1 Hydrogen bonds between the tyrosyl-RNA synthetase and tyrosyl adenylate. MC, main chain.

attempt to eliminate artefacts. The obvious experimental procedure is to crystallize the mutants and use protein crystallography to detect structural changes. Such a technique will detect changes of ~ 0.1 Å or more but will miss a series of smaller changes. (The effects of small relaxations of protein structure on binding are not known.)

Because kinetics measurements are exceptionally sensitive and can detect changes of a fraction of a kilocalorie, we use kinetic methods to increase the reliability of analysis. For example, application of the 'double-mutant' procedure of Carter *et al.*¹⁴ has shown that mutation of residues 35 and 48 does not cause structural changes propagated through the protein¹⁴. Experiments in which we have mutated side chains by the removal of methyl groups buried in the protein, however, show that these small changes can weaken binding by up to 1 kcal mol⁻¹ (A.J.W., A.R.F., P.C. and G.W., unpublished data). Because of this, we have analysed many different mutants to observe trends. The combination of the extensive and consistent kinetic data (see below) and modelling by molecular graphics suggests that the results so far reflect the direct effects of hydrogen bonding itself and are not dominated by structural change.

Hydrogen-bond strengths *in vacuo*

The stabilization energies of hydrogen bonds *in vacuo*, between compounds X-H and B-Y in equation (3), have been calculated¹⁵:

$$X-H \cdots B-Y = X-H + B-Y \quad (3)$$

(where -H is a hydrogen-bond donor and -B is an acceptor). Representative values for the energies of stabilization for different donors and acceptors are: water/water, -6.4 kcal mol⁻¹; water/CH₃SH, -3.1 kcal mol⁻¹ for -SH as the acceptor, -3.2 kcal mol⁻¹ for -SH as the donor; imidazolium/water, -14 kcal mol⁻¹; acetate/water, -19.8 kcal mol⁻¹. These values are much greater than those in Table 2 for the overall energies of bonding in aqueous solution.

General model for H bonding in water

Our data fit the formulation of Jencks¹⁶ and Hine¹⁷:



For convenience, we assume that the enzyme has a hydrogen-bond donor, -H, and the substrate (S) an acceptor, -B, that pair in the enzyme-substrate complex. In the free enzyme, -H and -B are bound to water molecules. The number and types of hydrogen bonds are conserved in the reaction, which is thus essentially isoenthalpic (within the limits of Hine's¹⁷ equation). For example, as discussed by Wilkinson *et al.*⁵, a -SH group is just as effective as a -OH group as a hydrogen-bond donor/acceptor, despite the different absolute strengths of -SH $\cdots$ O and -OH $\cdots$ O bonds in equation (3); if, in equation (4), -H is part of a -SH group, then there is a -SH $\cdots$ O bond

Table 1 Activation of tyrosine by tyrosyl-tRNA synthetase and its mutants

Enzyme	k_{cat} (s ⁻¹)	K_M (ATP) (mM)	K_M (Tyr) (μM)	k_{cat}/K_M (ATP) (s ⁻¹ M ⁻¹)	k_{cat}/K_M (Tyr) (s ⁻¹ M ⁻¹)
Wild type	8.35	1.08	2.23	7,730	3.74×10^6
Tyr → Phe 34	6.86	1.2	4.4	5,720	1.56×10^6
Cys → Gly 35	2.95	2.6	2.7	1,130	1.09×10^6
Cys → Ser 35	2.52	2.4	2.6	1,050	9.65×10^5
His → Asn 48	7.90	1.4	3.8	5,640	2.08×10^6
His → Gly 48	2.00	1.3	3.2	1,540	6.25×10^5
Thr → Ala 51	8.75	0.54	2.0	16,200	4.36×10^6
Gln → Tyr 195	0.19	2.5*	100	76*	1.90×10^3
Tyr → Phe 169†	6.05	1.25‡	1,030	4,840‡	5.88×10^3
Δ(321-419)†	7.50	1.08	2.4	6,940	3.12×10^6

Mutant enzymes were prepared, assayed and active-site titrated as described previously^{5,14}. Activation was measured by pyrophosphate exchange at 25 °C, pH 7.78 in the presence of 144 mM Tris-Cl, 10 mM MgCl₂ and 2 mM PP_i. Kinetic constants for variation of ATP were determined in the presence of 0.05 mM tyrosine (except where indicated otherwise) and for tyrosine in the presence of 2 mM ATP. Values of k_{cat} are extrapolated to infinite concentration of tyrosine and ATP.

* 0.3 mM tyrosine.

† Experiments on truncated enzyme with residues 321-419 deleted (the tRNA binding domain)²².

‡ 1.7 mM tyrosine.

on the left-hand side and a -SH $\cdots$ O bond on the right in the enzyme-substrate complex. The contribution of hydrogen bonds to enzyme-substrate binding energy arises from entropy; water hydrogen bonded to the enzyme or substrate has lower entropy than bulk water and the release of hydrogen-bonded water helps drive enzyme-substrate binding^{13,16}. Thus, there is a crucial distinction between the absolute energy of a hydrogen bond as in equation (3) and the overall energetics of hydrogen bonding in solution. It is the overall energetics that are important in the binding of enzymes and substrates, and it is the overall energetics that we measure from site-directed mutagenesis.

The removal of one of the hydrogen-bonding groups from equation (4), for example a side chain of the enzyme as in equation (5),



does not necessarily lead to the loss of the absolute binding energy (enthalpy) of a hydrogen bond as defined by equation (3). Deletion of a hydrophilic side chain means that a water molecule must be located next to a hydrophobic region of the enzyme in the space vacated. This water molecule is at a high energy because it is at an interface and not in bulk water where it can fulfil readily all its hydrogen bonding. There is no hydrogen bond from E to H₂O in the free enzyme and so this partly (or even fully) compensates for the lack of the hydrogen bond from E to -B in the enzyme-substrate complex. When the substrate binds, it displaces the high-energy water molecule (which returns to the bulk solvent), and the hydrophilic hydrogen-bond acceptor of the substrate occupies the 'hydrophobic' site. The overall energetics of the reaction in equation (5) depend on the precise interactions made by the mutant enzyme with the water molecule and the group -B on the substrate. If, for example, the enzyme-bound water molecule in equation (5) is in the same orientation as that in equation (4), then equation (5) may be written as



There is one hydrogen bond on the right and a similar one on the left of equation (6). This reaction is approximately isoenthalpic when -B is -OH and, to a first approximation, the energetics of equation (6) are the same as those of equation (4). Therefore, deletion of the hydrogen bond in the enzyme-substrate complex does not lead to the overall loss of the absolute strength of a hydrogen bond and may, in certain circumstances, cause no loss of binding energy. The energetics clearly depend on the precise nature of the interactions in each particular example and so will

Table 2 Comparative binding energies of wild-type and mutant enzymes with substrates

Comparison (Residue and position)		Substrate	ΔG (kcal mol ⁻¹)
Phe 34	Tyr 34*	Tyr	0.52
Gly 35	Cys 35*	ATP	1.14
Ala 51	Cys 51	ATP	0.47
Gly 48	Asn 48	ATP	0.77
Gly 48	His 48*	ATP	0.96
Ser 35	Cys 35*	ATP	1.18
Phe 169†	Tyr 169†	Tyr	3.72
Gly 195	Gln 195*	Tyr	4.49
Gly 35	Ser 35	ATP	-0.04
Ala 51	Thr 51*	ATP	-0.44

The first two columns show the residues compared. The apparent contributions (ΔG) of the side chains of different amino acids to the binding energy of the enzyme-transition state complexes were calculated by comparing the ratios of k_{cat}/K_M for activation by wild-type and mutant enzymes as described previously⁵, using the equation $\Delta G = -RT \ln \{(k_{cat}/K_M)_{mut}/(k_{cat}/K_M)_{wt}\}$ (subscripts mut and wt refer to mutant and wild-type (or reference) enzymes respectively).

* Wild-type; † truncated enzyme²².

Table 3 Activation of tyrosine and phenylalanine by tyrosyl-tRNA synthetases

Enzyme	Activation of Tyr			Activation of Phe	$\frac{(k_{cat}/K_M)_{Tyr}}{(k_{cat}/K_M)_{Phe}}$
	k_{cat} (s ⁻¹)	K_M (μM)	k_{cat}/K_M (s ⁻¹ M ⁻¹)	k_{cat}/K_M (s ⁻¹ M ⁻¹)	(Relative specificity)
Wild type	5.4	2.2	2.5 × 10 ⁶	17	1.5 × 10 ⁵
Tyr → Phe 34	4.4	4.4	1.0 × 10 ⁶	100	1.0 × 10 ⁴

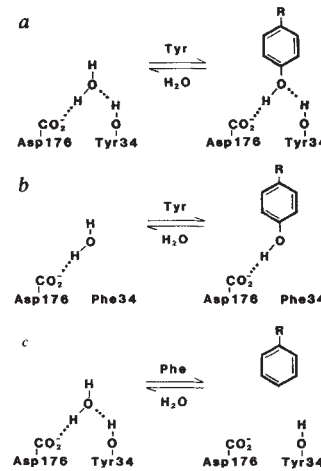
Impurities of tyrosine in the phenylalanine were removed by scavenging with tetranitromethane²³. Conditions as described in Table 1 but data are not extrapolated to infinite concentration of ATP but given for 2 mM ATP. For activation of phenylalanine, the Phe concentration was varied between 5 and 30 mM, well below the K_M value. The specificity of wild-type enzyme is close to that measured previously under different conditions²³.

vary from case to case. Before this study, the magnitudes of the energetic changes were unknown. Now we discuss these and show how particular examples may be rationalized and categorized.

Case A. Deletion of a side chain (-H) that interacts with an uncharged group on the substrate. Equation (5) or (6) describes this. The low contribution to specificity of a hydrogen bond between two uncharged residues is exemplified by the mutation Tyr → Phe 34 (Figs 1, 2). This is a very conservative mutation whereby a small group is deleted whose interaction is important in the recognition of tyrosine compared with phenylalanine. Yet, K_M for tyrosine increases by a factor of only two (Table 1) and the specificity for tyrosine decreases by a factor of only 15 (Table 3). The apparent bond energy of 0.5 kcal mol⁻¹ is one of the lowest yet found, perhaps because the tyrosine -OH is a poorer hydrogen-bond acceptor than an aliphatic or water -OH and so the bond from Tyr 34 to the tyrosyl -OH of the substrate is relatively weak. The thiol-containing side chain from Cys 35, which donates to the 3'-OH of the ribose of ATP, contributes 1.1 kcal mol⁻¹. The tyrosyl-tRNA synthetase from *Bacillus caldovenax* differs from the enzyme from *B. stearothermophilus* by only four amino-acid replacements (M.D. Jones, unpublished data). One of these is an asparagine at position 48. The side chain of Asn 48 contributes 0.77 kcal mol⁻¹ and that of His 48 contributes slightly more binding energy at 0.96 kcal mol⁻¹.

Note that equation (5) is relatively insensitive to the nature of the side chain deleted (when -B is uncharged). For example, consider the deletion of a charged histidine side chain (-H is imidazolium) as in the mutation His → Gly 48. Then, relative to wild-type enzyme, a strong interaction between imidazolium

Fig. 2 Illustration of the hydrogen-bond inventory on tyrosine and phenylalanine binding to the tyrosyl-tRNA synthetase. *a*, Binding of wild-type enzyme and tyrosine involves the formation of two hydrogen bonds between its hydroxyl and Asp 176 and Tyr 34, but two equivalent hydrogen bonds of Asp 176 and Tyr 34 with water are broken. *b*, Binding of tyrosine to the Tyr → Phe 34 mutant involves just the formation of one hydrogen bond with the enzyme, but only one hydrogen bond is broken on displacing the bound water molecule. *c*, The binding of phenylalanine to the enzyme with the concomitant displacement of water would lose a strong hydrogen bond between water and Asp 176 and lead to a partially desolvated charge between the enzyme and substrate. Also, Tyr 34 would be unsolvated. Further, when tyrosine is bound, there are van der Waals' interactions between its phenolic hydroxyl and the enzyme, which are not present when phenylalanine is bound. It is probable that phenylalanine binds to the enzyme with the water molecule remaining bound between Asp 176 and Tyr 34. Note that in *a* and *b* the water molecules released from the enzyme on the binding of tyrosine can be considered to take the place of the tyrosine hydroxyl in solution so that there is no net change in the number of hydrogen bonds in solution.

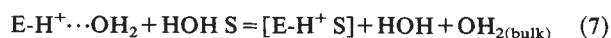


and the substrate is lost in the mutant enzyme-substrate complex, but this is compensated for by a loss of a strong interaction between imidazolium and water in the free mutant enzyme.

We have analysed several mutants as well as those listed in Table 2. In all cases, the deletion of a side chain that interacts with an uncharged group on the substrate loses between 0.5 and 1.5 kcal mol⁻¹ of binding energy.

Case B. Deletion of a side chain that interacts with a charged group on the substrate. This is exemplified by the mutation Tyr → Phe 169 (Fig. 1) whereby the hydrogen bond to the ammonium ion is lost in the enzyme-substrate complex. Equation (5) still describes the situation, but there is now the loss of a strong hydrogen bond between the charged group on the substrate and water when forming the enzyme-substrate complex and 3.7 kcal mol⁻¹ of binding energy are lost on Tyr → Phe 169. Gln 195 binds to a carboxylate oxygen of tyrosine in the E.Tyr complex and to the carbonyl oxygen of tyrosyl adenylate (Fig. 1; refs 6, 7, 18). On mutation of Gln → Gly 195 there is a loss of binding energy of 4.5 kcal mol⁻¹ in the transition state where the oxygen atom is fractionally charged.

Case C. Deletion of a group on the substrate that interacts with a charged residue on the enzyme, described by the equation.



This has similar consequences to Case B. A strong interaction in the enzyme-water complex is lost, an example being the 'modification' of tyrosine to phenylalanine. Phenylalanine binds more poorly to the enzyme by ~7 kcal mol⁻¹ (Table 3). The phenylalanine either displaces the water molecule that is bound between Asp 176 and Tyr 34 in the enzyme (Fig. 2), leaving two unpaired hydrogen bonds, or, more probably, binds with a water molecule still bound between Asp 176 and Tyr 34. The water is crammed in a space which is too small, so that there are unfavourable interactions with the substrate.

A minimum estimate of the strength of the bond between Asp 176 and the tyrosine -OH can be made from the relative binding of tyrosine and phenylalanine to the mutant Tyr → Phe 34 (5.45 kcal mol⁻¹ calculated from the data in Table 3). If phenylalanine displaces the bound water molecule as in Fig. 2, then there will be the loss of the hydrogen bond and also of the dispersion energy between the enzyme and the -OH group. The latter is worth ~1-2 kcal mol⁻¹ (ref. 19) so the hydrogen bond

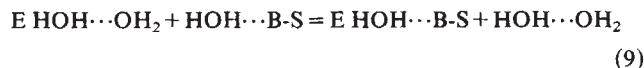
is worth 3.5–4.5 kcal mol⁻¹. If it is easier to bind phenylalanine without displacing the water, then the bond must be stronger than this.

Case D. Deletion of a side chain when there are geometrical constraints against hydrogen bonding in an enzyme–substrate complex. The above cases assume that there are no constraints on forming hydrogen bonds in the enzyme–substrate complex. When, as in equation (8), there is poor hydrogen bonding



because the intermolecular distance between –H and –B is too great, there is just one good hydrogen bond on the right-hand side but two on the left of the equation. Deletion of –H may increase the affinity of the enzyme for the substrate because with the mutant enzyme there is one good hydrogen bond on the right but now only one on the left. Mutation of Thr→Ala 51 (ref. 12) is an example of this, as is Ser→Gly 35.

Case E. Deletion of a side chain on the enzyme or a group on the substrate that allows access of water between enzyme and substrate in their complex. It was suggested in Case C that phenylalanine binds to the tyrosyl-tRNA synthetase without displacing the bound water molecule but with unfavourable van der Waals' repulsion. On deletion of a large side chain from the enzyme, there could perhaps be adequate room for a water molecule to occupy comfortably the gap left by the deleted side chain, as in equation (9).



A hydrogen-bond inventory shows that the number of hydrogen bonds may be formally conserved, as in Case A, equation (6). The energetics are expected to be similar to Case A for –B being an uncharged side chain. When –B is charged, as in Case B, the retention of solvent water by the charged group in the mutant enzyme–substrate complex will tend to attenuate the effects described in Case B.

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1. Kauzmann, W. *Adv. Protein Chem.* **14**, 1–63 (1959).
2. Klotz, I. M. & Franzen, J. S. *J. Am. chem. Soc.* **84**, 3461–3466 (1962).
3. Cantor, C. R. & Schimmel, P. R. *Biophysical Chemistry* pt 1, 277 (Freeman, San Francisco, 1980).
4. Fersht, A. R. & Jakes, R. *Biochemistry* **14**, 3350–3356 (1975).
5. Wilkinson, A. J., Fersht, A. R., Blow, D. M. & Winter, G. *Biochemistry* **22**, 3581–3586 (1983).
6. Monteilhet, C. & Blow, D. M. *J. molec. Biol.* **22**, 407–417 (1978).
7. Rubin, J. & Blow, D. M. *J. molec. Biol.* **145**, 489–500 (1981).
8. Bhat, T. N., Blow, D. M., Brick, P. & Nyborg, J. *J. molec. Biol.* **158**, 699–709 (1982).
9. Blow, D. M. & Brick, P. in *Biological Macromolecules and Assemblies* Vol. 2 (eds Jurnak, F. & McPherson, A.) 442–469 (Wiley, New York, 1985).
10. Fersht, A. R. *et al. Angew. Chem.* **23**, 467–473 (1984).

Biological specificity

The classical view of biological specificity is that binding energy is provided by dispersion forces and the hydrophobic effect, whilst complementary hydrogen bonds and salt bridges give specificity^{16,20}. This study shows that, irrespective of any small structural effects that occur on deletion of side chains, a side chain that forms a hydrogen bond between the enzyme and an uncharged group on a substrate provides only 0.5–1.5 kcal mol⁻¹ of binding energy relative to the absence of the side chain. This means that an unpaired uncharged hydrogen bond provides a factor of only 2.5–15 or so towards specificity. But the absence of a group on the enzyme or a substrate that should form a hydrogen bond with a charged group (as in Cases B and C) affects binding by ~4 kcal mol⁻¹ and is worth a factor of 1,000 in specificity. Thus, specificity is caused to some extent by hydrogen bonding but is best mediated by charged residues.

In principle, the same reasoning may be applied to the contribution of hydrogen bonding in base pairing during DNA replication or transcription. There is, however, one important difference when analysing, for example, G·T and A·C mispairing. All the cases discussed here concern the removal of groups so that no unfavourable steric interference is set up between the enzyme and substrate. But, if in base mispairing, G·T takes up the same overall geometry as an A·T base pair and A·C the same as a G·C pair, unfavourable steric and electrostatic interactions will be set up between >NH groups. These effects will compound the loss of hydrogen bonds in the mispairs. In general, unfavourable steric interactions are an important element in specificity, as van der Waals' repulsion energies are such a strong function of interatomic distance. High specificity by steric repulsion is the basis of double-sieve sorting of amino acids by the aminoacyl-tRNA synthetases²¹. Thus, two important components of biological specificity are the avoidance of unsolvated charges and unfavourable steric interactions between enzyme and substrate.

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11. Winter, G., Fersht, A. R., Wilkinson, A. J., Zoller, M. & Smith, M. *Nature* **299**, 756–758 (1982).
12. Wilkinson, A. J., Fersht, A. R., Blow, D. M., Carter, P. & Winter, G. *Nature* **307**, 187–188 (1984).
13. Fersht, A. *Enzyme Structure and Mechanism* Ch. 2 (Freeman, New York, 1985).
14. Carter, P. J., Winter, G., Wilkinson, A. J. & Fersht, A. R. *Cell* **38**, 835–840 (1984).
15. Weiner, S. J. *et al. J. Am. chem. Soc.* **106**, 765–784 (1984).
16. Jencks, W. P. *Catalysis in Chemistry and Enzymology* (McGraw-Hill, New York, 1969).
17. Hine, J. *J. Am. chem. Soc.* **94**, 5766–5771 (1972).
18. Monteilhet, C., Blow, D. M. & Brick, P. *J. molec. Biol.* **173**, 477–485 (1984).
19. Fersht, A. R. & Dingwall, C. *Biochemistry* **18**, 1245–1249 (1979).
20. Fersht, A. R. *Trends biochem. Sci.* **9**, 145–147 (1984).
21. Fersht, A. R. & Dingwall, C. *Biochemistry* **18**, 2627–2631 (1979).
22. Wayne, M. M. Y., Winter, G., Wilkinson, A. J. & Fersht, A. R. *EMBO J.* **2**, 1827–1829 (1983).
23. Fersht, A. R., Shindler, J. S. & Tsui, W.-C. *Biochemistry* **19**, 5520–5524 (1980).

LETTERS TO NATURE

New quasars with $z = 3.4$ and 3.7 and the surface density of very high redshift quasars

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It has been suggested that there is a sharp decline in the space density of quasars beyond redshifts $z \sim 3.5^1$. We describe here the discovery on a UKST (UK Schmidt telescope) low-dispersion IIIaF objective prism plate of a QSO (quasistellar object) with $z = 3.7$, which is the second highest redshift QSO known and, moreover, lies in the same region in which a QSO with $z = 3.61$ has already been reported². We also note a QSO with $z = 3.4$. Before these

observations, only seven QSOs with $z \geq 3.4$ were known throughout the whole sky. When compared with the results of a search for QSOs with $2.7 < z < 3.3$ on the same plate, these observations do not support suggestions of a $z \sim 3.5$ cut-off in the QSO distribution¹, but rather support the suggestion of a steady decline from $z \sim 2$. We show here that deep searches over small areas of sky are unlikely to be successful in discovering QSOs at $z > 4$ and that such searches must be carried out over very large areas, even if only to a moderate limiting magnitude of $m(R) \sim 19$.

The UKST, in combination with the 44-arc min objective prism and IIIaJ plates, has proved a powerful tool for the detection of QSOs by their Ly α emission up to $z = 3.3$, a limit set by Ly α then passing beyond the red limit of the IIIaJ response at $\sim 5,200 \text{ \AA}$. This particular prism has usually been considered to have too low a dispersion to be used effectively with a IIIaF emulsion, which has a red limit at $\sim 6,800 \text{ \AA}$ and can, in principle, detect QSOs up to $z \approx 4.5$. However, the discovery of the $z = 3.52$ QSO 1159+123 (ref. 3) has shown that UKST IIIaF prism plates can be used to find high- z QSOs; the selection problems caused by irregularities in the spectral response of the

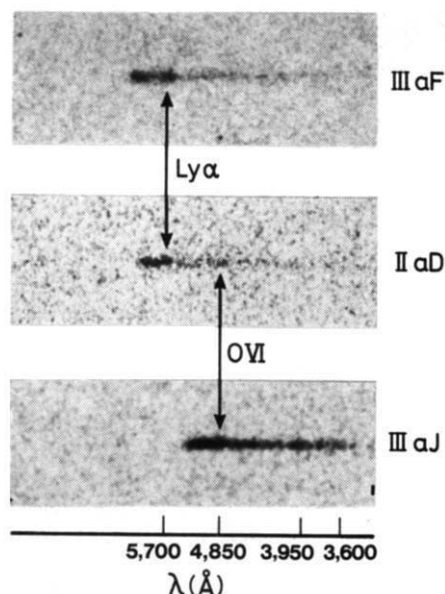


Fig. 1 Objective prism spectra of 0055-2659 reproduced by courtesy of the UK Schmidt Telescope Unit.

IIIaF emulsion can be minimized by using auxiliary IIaD and IIIaJ prism plates to check selected candidates. The rapid decrease of the prism dispersion with increasing wavelength produces in the majority of IIIaF prism spectra a strong enhancement of the continuum emission near their red limits. This makes emission lines above 6,000 Å difficult to distinguish and limits the maximum redshift attainable to $z \approx 3.9$. As QSOs with $z \leq 3.3$ are better found on IIIaJ plates, the useful range of the IIIaF plates is $3.3 \leq z \leq 3.9$.

The new QSOs were discovered on a set of high-quality plates centred on RA 00 h 53 min, dec. $-28^\circ 03'$, the same field in which the $z = 3.61$ QSO DHM0054-284 (ref. 2) was found. Their positions, magnitudes and estimated redshifts are given in Table 1. These were the only new objects found in our search about which we were reasonably certain on the basis of the prism data alone, although we would certainly have included DHM0054-284 in Table 1 if it had not already been identified. The objective prism spectra of the $z = 3.7$ QSO 0055-2659 are reproduced in Fig. 1 and illustrate how the IIaD and IIIaJ spectra are used to support the identification. The Ly α emission visible in both the IIIaF and IIaD spectra, the OVI emission visible in the IIaD and IIIaJ spectra and the heavy absorption in the IIIaJ continuum, all combine to support the identification as a high- z QSO. Our measurements of the various emission features are presented in Table 2 and the estimated redshifts are in such good agreement that we had no hesitation in confirming from the prism data alone the identity of 0055-2659 as a QSO with $z = 3.7 \pm 0.05$. Its strong blue continuum, extending to at least 800 Å in the rest frame, makes this bright QSO particularly suitable for ground-based absorption line studies and for satellite observations. The $z = 3.4$ QSO 0042-2627, could also be identified with confidence. The emission line in the IIIaF spectrum is too strong to be an artefact of the emulsion response and it is just visible at the extreme limit of the IIIaJ spectrum.

Observations by A. Boksenberg (personal communication) on the 2.5-m Isaac Newton telescope on La Palma have already

confirmed the estimated redshift for 0055-2659, and those by A. Boksenberg and W. L. W. Sargent (personal communication) on the Palomar 5-m telescope, that of 0042-2627. The measured redshifts are 3.67 and 3.4, respectively, which establishes 0055-2659 as second in redshift only to the $z = 3.78$ radio-selected QSO 2000-330 (ref. 4) and as the highest redshift QSO selected by purely optical techniques. Of the nine known QSOs with $z \geq 3.4$, three have now been discovered in the 36 square degrees of one UKST field, a success rate that raises questions regarding the failure of previous high- z QSO surveys going to fainter magnitude limits.

The failure to find any QSOs with $z > 3.8$ in the earlier special searches over areas of only a few square degrees must be considered in light of the fact that even in the redshift range $3 < z \leq 3.3$ QSOs are not exactly common. Although even weak emission-line QSOs in this redshift range are readily found on Schmidt Telescope IIIaJ prism plates, and such plates covering several thousands of square degrees have now been searched, the latest QSO catalogue⁵ lists only 38 in this redshift interval. As far as we know, the highest number of QSOs with $3 < z \leq 3.3$ on a single Schmidt plate has been found by C.H. and W. L. W. Sargent (unpublished data) on a UKST plate centred on RA 15 h 00 min, dec. $+11^\circ 00'$. They have confirmed by slit spectroscopy a total of nine such QSOs with $B(J) < 20$, with only a few candidates remaining to be examined so far. This represents a decrease by a factor of at least 10 from the number of QSOs they found around $z = 2$ in the same redshift interval. Hazard⁶ claims that this steep decrease cannot be attributed to a bias against the selection of the higher- z QSOs but corresponds to a decrease in the co-moving density of emission-line QSOs for $z \geq 2$. He further suggests that the failure to detect QSOs around $z = 4$ over small areas of sky, rather than implying a QSO cut-off at $z \sim 3.5$, represents an extrapolation of the observed decline between $z = 2$ and $z = 3$ to higher redshifts.

Assuming $m(J) - m(R) \approx 0.7$ for QSOs around $z = 3$ (see Fig. 1 in ref. (2)) and a rate of decrease of a factor of 10 per unit redshift interval, the extrapolation gives an expected six QSOs per UKST field with $m(R) < 19.3$ and $3.3 < z < 3.9$. The discovery of three QSOs in the 0053-2803 field with $m(R) \leq 18$ is consistent with this estimate, particularly as some of the $z > 3$ QSOs in the 1500+1100 field are weak emission line objects that would probably not have been detected on the IIIaF plate. The only surprise, perhaps, is that all are so bright, as, for the usual power law luminosity function, the majority would be expected to lie near the plate limit at about 19 mag; we have searched carefully for fainter candidates, but without success, apart from a possible weak line $z = 3.5$ QSO with $m(R) = 18.4$ at RA 01 h 01 min 15.7 s, dec. $-26^\circ 53' 26''$.

Any significant clustering of high- z QSOs or any difference in the redshift distribution in different regions of sky could invalidate the above comparison. We have, therefore, attempted a direct comparison of the QSO densities between $3.3 < z < 3.9$ and $z \sim 3$ by searching the IIIaF plate for QSOs with $2.7 < z < 3.3$ using the same procedures adopted in the search for the higher-redshift objects. Genuine QSOs with $z < 3.3$ can be unambiguously identified by their Ly α emission in the IIIaJ spectra. We did, indeed, find that not all the $z \sim 3$ QSOs that are recognizable on the IIIaJ plates can be found on the IIIaF plates, showing that our survey for objects with $z > 3.3$ is also probably incomplete. The search yielded a total of 11 QSOs with $2.7 < z < 3.3$. When compared with our results for $3.3 < z < 3.9$, this represents, for a magnitude-limited sample and for $\Delta z = +0.6$, a decrease in QSO density by a factor of 3, which is in satisfactory agreement

Table 1 Positions, magnitudes and estimated redshifts for new QSOs

	z	(h)	RA min	s	(°)	dec. '	"	$m(R)^*$	$M(R)^{\dagger}$
0042-2627	3.4	00	42	06.2	-26	27	43	~ 17	~ -31.7
0055-2659	3.7	00	55	32.5	-26	59	25	17.2	-31.8
(DHM0054-284)									-31.2)

* UKST R IIIaF emulsion, RG 630 filter (measured on Cambridge APM).

$\dagger$ Calculated assuming a Hubble constant, $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, deceleration in parameter $q_0 = 0$.

Table 2 Emission features of QSO 0055-2659

Emulsion	λ (Å)	Identification	z
IIIaF	5,700	Lyman- α	3.70
IIaD	5,690	Lyman- α	3.69
IIIaJ	4,875	Ovi	3.71

Adopted redshift 3.70 ± 0.05

with the decrease by a factor of ≥ 10 , between $z=2$ and $z=3$ found on the 1500+1100 plate. These results are more consistent with a steady decline in the QSO density above $z>2$ than with a cut-off in the QSO distribution at $z\sim 3.5$. This could imply that the birth of the majority of QSOs occurred at $z\sim 2$ and could have important implications regarding the epoch of galaxy formation and the rate of growth of the massive galactic nuclei that are generally believed to be the primary QSO energy sources.

Our results, relating both to the fall in the surface density of QSOs with increasing redshift and to the apparent lack of a strong magnitude dependence in the number of high- z QSOs, are consistent with the type of combined luminosity and density evolution proposed by Koo⁷. According to Koo, the luminosity function at $z=2.15$ corresponds to a steep power law dependence at the bright end with the QSO density rising rapidly with decreasing brightness up to $M_B = -28$, where there is a transition to a much flatter distribution for the fainter objects. With increasing redshift the basic form of the distribution remains unaltered, but it is displaced in the $\log \rho/M$ plane so that the turn-over point shifts both to brighter absolute magnitudes and to lower densities. For sufficiently high redshifts the observed QSOs will be dominated by those lying in the flat region between the turn-off point and the absolute magnitude corresponding to the limiting apparent magnitude. The occurrence of a bright high- z QSO in a given field is thus as probable as a QSO near the limiting magnitude, although, in practice, the former is the one more likely to be selected; that all known high- z QSOs are relatively bright is therefore no accident. In a magnitude-limited sample, the number of QSOs in a given redshift interval will decrease with increasing redshift at a rate approximately corresponding to the degree of density evolution, but modified by the different rates of displacement of the turn-off point and the limiting magnitude; if there is a maximum QSO luminosity, the numbers will decrease more rapidly when it comes into effect.

It is easy to show, by a similar extrapolation to that used earlier, that the expected number of QSOs to $m(R)\sim 21$ with $4<z<5$ is less than 0.1 per square degree, a figure quite consistent with the reported null detections over an area of 5 square degrees¹. Furthermore, this figure is only weakly dependent on the magnitude limit and about half of the QSOs will be brighter than 19 mag. Deeper surveys cannot be used to compensate for a drastically reduced search area and it follows that searches for very high- z QSOs, carried out over small areas of sky, have a very low probability of success. Searches over very large areas, even if only to a moderate limiting magnitude of $m(R)\sim 19$, offer the only realistic hope.

We have demonstrated the usefulness of the UKST low-dispersion IIIaF objective prism plates in high redshift surveys, at least up to $z\sim 3.7$. The extension to $z>4$ will probably require the use of the new higher-dispersion prism with appropriate filters or the use of broad-band colours. The strong Ly α emission of all presently known high- z QSOs has at least removed the worry that increasing absorption due to intervening gas or dust might prevent their discovery when using slitless spectroscopy techniques. Finally, the observations presented here should encourage more active searches for these objects which provide the only means we have at present for probing the early history of the Universe.

We thank the UKST for help and for excellent plate material and R. Terlevich for his part in the development of the Cambridge objective prism programme. We also thank M. Irwin for providing the magnitude measurements from the Cambridge APM, R. Sword for the reproduction of the objective prism spectra and Dr D. C. Koo for useful discussions.

Infrared Seyferts: a new population of active galaxies?

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A Seyfert spectrum is one of the most important indicators of nuclear activity in galaxies. It is characterized by broad emission lines with high ionization. Most known Seyferts have been found indirectly, for example, by the presence of a strong ultraviolet continuum (Markarian galaxies) or of intense radio emission (broad/narrow-line radio galaxies). Here, we present a new method for finding hitherto unknown Seyferts based on their mid-infrared spectra and we show using data from the Infrared Astronomical Satellite (IRAS) that this method has a success rate of $\sim 70\%$. One Seyfert, so discovered, contains the most luminous infrared nucleus known so far. X-ray surveys are also an efficient means of finding new Seyferts and QSOs (quasistellar objects)^{1,2}, but tend to reveal samples at considerably higher redshifts and lower space density. We believe that 'infrared' Seyferts may soon outnumber those detected by other methods.

IRAS³ has detected many thousands of galaxies, principally at wavelengths of 60 and 100 μm . Most galaxies detected in flux-limited samples at these wavelengths^{4,5} have relatively steep infrared spectra with indices $\alpha\sim -2.5$ (defined by $S_\nu\propto\nu^\alpha$, where S_ν is the flux density at frequency ν). This cold infrared emission (~ 30 K) is believed to be produced in the disks of spiral and interacting galaxies⁵⁻⁷.

On the other hand, for most known cases of infrared emission from the nuclear region of active galaxies, the spectrum is significantly flatter in the infrared. For example, NGC1068 has a 250 K mid-infrared component whose spectrum peaks near 30 μm ⁸⁻¹¹ and which originates from a region ~ 60 pc in size. A similar component is observed in 3C390.3¹². Quasars also have relatively flat infrared spectra¹³.

These considerations prompted us to investigate whether a relatively flat infrared spectrum may be an indicator of nuclear activity. We selected a subsample of 'warm' objects from the partially completed IRAS survey, all at high galactic latitude ($|b|>20^\circ$) and with spectral indices between 25 and 60 μm of $-1.25<\alpha<-0.5$. These wavelengths were chosen as the most efficient for indicating flat-spectrum nuclear infrared emission. A cold galactic disk would dominate the 100–60- μm spectrum, possibly masking a warm nuclear component.

The IRAS sources so defined were identified using the Palomar Sky Survey and ESO/SRC (European Southern Observatory/Science Research Council) plates. After excluding objects that were clearly galactic (stars, small H II regions, planetary nebula), the remainder (54 objects) were published in IRAS Circular 11 as a list of candidate active nuclei¹⁴.

Spectra were taken of 40 of these objects (Table 1). The observations were made in photometric weather between 7 and 9 March 1984 with the Image Dissector Scanner (IDS) on the Boller and Chivens spectrograph at the 3.6-m ESO telescope at La Silla with a dispersion of 172 $\text{\AA mm}^{-1}$. This resulted in a wavelength coverage of 3,800–7,000 $\text{\AA}$, with a resolution of 11- $\text{\AA}$ FWHM. The exposure times for the objects discussed in this letter was 10 min. Wavelength calibration, flatfielding and flux calibration were carried out in the standard way with the ESO IHAP system (Image Handling and Processing), using the white dwarfs W485 and L745–46A as standard stars.

Our results are summarized in Table 1. In classifying the optical spectra, we adopt the scheme of Baldwin *et al.*¹⁵ in which

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1. Osmer, P. S. *Astrophys. J.* **253**, 28–37 (1982).
2. Shanks, T., Fong, R. & Boyle, B. J. *Nature* **303**, 156–158 (1983).
3. Hazard, C. *et al. Mon. Not. R. astr. Soc.* **211**, 45p–50p (1984).
4. Peterson, B., Savage, A., Jauncey, D. & Wright, A. *Astrophys. J.* **260**, L27–L29 (1982).
5. Veron-Cetty, M.-P. & Veron, P. *ESO Sci. Rep.* **1** (1984).
6. Hazard, C. in *Proc. Manchester Conf. Active Galactic Nuclei* (in the press).
7. Koo, D. C. *Proc. 24th Liège IAU Colloquium*, 240–244 (1983).

Table 1 Results of the spectroscopic observations

Name	Infrared spectrum	Redshift	[OIII] linewidth FWHM (km s ⁻¹)	Optical spectrum
0513-235P11		0.035:		No emission lines
0521-122P11	F1	0.0494	640	Seyfert I
0531-206P11	F2	0.0393		Strong H α
0556-348P11		0.032:		No emission lines in both components
0611-326P11	F1	0.0500	630	Seyfert II
0815+035P11		0.030:		Strong H α
0818+033P11		0.009:		No emission lines
1036-190P11		—		No emission lines
1051-273P11	F1	0.1606	720	Seyfert II
1105-115P11	F1	0.0545	780	Seyfert II
1119+045P11	F1	0.0386		Strong H α
1121-281P11	F1	0.0136	750	Seyfert II
1246-111P11	F1	0.0483	950	Seyfert II
1249-131P11		0.0138	370	Seyfert I
1304-234P11	F1	0.0092	550	Seyfert II
1305-241P11	F1	0.0137	580	Seyfert II
1316-242P11		0.0322		Weak H α (N-W component)
		0.035:		Strong H α (S-E component)
1319-164P11	F1	0.0167	1250	Seyfert II
1320-342P11		—		No emission lines
1329+022P11	F2	0.0863	1120	Seyfert II
1331-301P11	F2	0.0344		Strong H α
1331-234P11		0.0086	530	Seyfert II
1331-231P11		0.0446	870	Seyfert II (E-component has no emission lines)
1333-340P11	F1	0.0077	370	Seyfert I
1354-203P11	F2	0.0360		Strong H α (S component has no emission lines)
1356-188P11		0.0315		Strong H α
1402-316P11		0.093		No emission lines
1404+012P11	F2	—		No emission lines
1423-116P11		0.0419	<200	Nuclear HII region
1428-030P11		0.0436		Strong H α (N component)
		0.044:		Weak H α (S component)
1431-326P11	F1	0.0255	690	Seyfert II
1444-219P11	F2	0.0107		Strong H α
1458-222P11		0.045:		Weak H α (W component has no emission lines)
1509-211P11	F1	0.0442	520	Seyfert I
1524+007P11	F1	0.0506	480	Seyfert II
1548-037P11	F1	0.0301	620	Seyfert II
1618+068P11	F2	—		No emission lines
1832-594P11	F1	0.0192	950	Seyfert II
1840-624P11	F1	0.0135	480	Seyfert I
1844-532P11		0.0185	280	Seyfert II

Column 1 lists the IRAS designation as given in IRAS Circular 11. Column 2 codes for the shape of the infrared spectrum ($\alpha > -1.25$): F1 marks a flat spectrum between 25 and 60 μm ; F2 denotes that the 25- μm flux is unreliable, but that the spectrum is flat between 60 and 100 μm . The measured redshifts are given in column 3 (the error is smaller than 0.001, except for those marked ':'). The corrected [OIII] linewidths (FWHM deconvolved with the instrumental response) are given in column 4 and the typical uncertainty is 60 km s⁻¹. Column 5 gives the type of optical spectrum and information about the number of optical counterparts possibly associated with the infrared sources.

Seyfert galaxies have high ionization with [OIII] λ 5007/H β > 6 and [NII] λ 6584/H α > 0.4. All the spectra defined in this way have broad resolved [OIII] lines >250 km s⁻¹ full-width to half maximum (FWHM), also typical of Seyferts¹⁶⁻¹⁸. Although higher resolution spectra are clearly necessary, the results obtained up to now suggest that on average the widths of the forbidden lines are substantially larger (57% broader than 600 km s⁻¹) than in previous surveys¹⁹ (15% broader than 600 km s⁻¹), which may be an effect of the large fraction of Seyfert IIs in this sample, as they are known to have broad forbidden lines¹⁶.

One of these infrared Seyferts (Fig. 1) is IRAS1051-273P11. Our measured redshift of 0.161 combined with the IRAS 25- μm

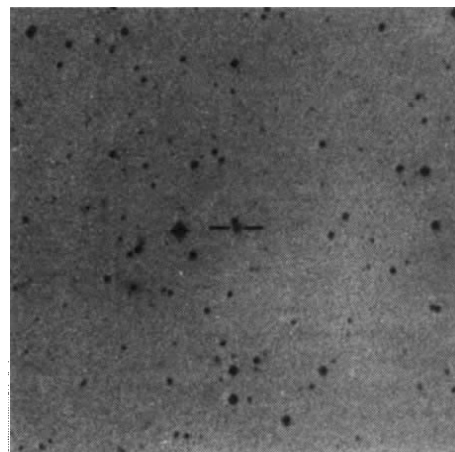


Fig. 1 A reproduction of the ESO/SRC J-plate of IRAS1051-273P11. North is up, east to the left; the picture encompasses 5 $\times$ 5 arc min on the sky. Close to the north of this Seyfert galaxy is a star.

flux density of 0.4 Jy results in a 25- μm luminosity of $10^{25.3} \text{ W Hz}^{-1}$, which is an order of magnitude larger than that of the mid-infrared component of either NGC1068 or 3C390.3, making it the most luminous Seyfert galaxy known in this wavelength range. If the 25- μm flux is thermal in origin, arguments analogous to that used for NGC1068¹¹ would require heating by an ultraviolet source of $2 \times 10^{12} L_{\odot}$.

In Table 1, 24 objects have reliably-determined flat infrared spectra between either 60 and 25 μm or between 60 and 100 μm . Of these flat-spectrum objects 17 (that is, 71%) have Seyfert spectra, which should be compared with a Seyfert rate of $\sim 10\%$ for Markarian objects. Hence a flat infrared spectrum is a remarkably efficient predictor of the Seyfert phenomenon and a preliminary analysis of IRAS circular 16 (ref. 20), which contains sources with similar infrared colours, seems to confirm this picture. Also, the IRAS source 0421+040P06^{21,22}, which has a 25-60- μm spectral index within our selected range, has been found recently to have a Seyfert-type spectrum.

Extrapolation of our results to the all-sky survey indicates that the IRAS database contains several thousand Seyferts, compared with ~ 330 Seyferts previously known²³. Taking the redshifts of the 21 Seyferts in Table 1 and assuming a Hubble constant of 75 km s⁻¹ Mpc⁻¹, the space density of the infrared Seyferts, determined using the V/V_{max} method, is $1.7 \times 10^{-4} \text{ Mpc}^{-3}$, 2.5 times greater than values based on the Markarian Seyferts²⁴. As no correction was made for incompleteness, this should be viewed as a lower limit. The fractional error based on this sample is 40%, but combination with recent new data yields the same figure with a 25% uncertainty.

Seyferts found through X-ray surveys are quite distinct in their properties from the infrared Seyferts; the space density of the X-ray objects is more than an order of magnitude lower and they are on average 10 times further away. Indeed a large fraction of them may more properly be called QSOs.

How do the infrared Seyferts differ from the Markarian Seyferts and why have they not been discovered before? The possibility that, using infrared criteria, we select more distant galaxies than does the Markarian method is improbable as there is no significant difference in the redshift distribution of our sample compared with the Markarian one²⁴ and both have median redshifts of 0.03. It is more probable that the infrared Seyferts are systematically fainter in the optical than the Markarian Seyferts²⁴. The Markarian catalogue becomes incomplete below an apparent magnitude of 15.5; although there is considerable uncertainty in the brightness of the infrared Seyferts, our initial estimates indicate that several are fainter than this magnitude.

It is also not unexpected that a large number of infrared Seyferts would be missed by the Markarian catalogue. The same dust that is presumably responsible for the infrared emission would significantly redden the non-thermal visible light from

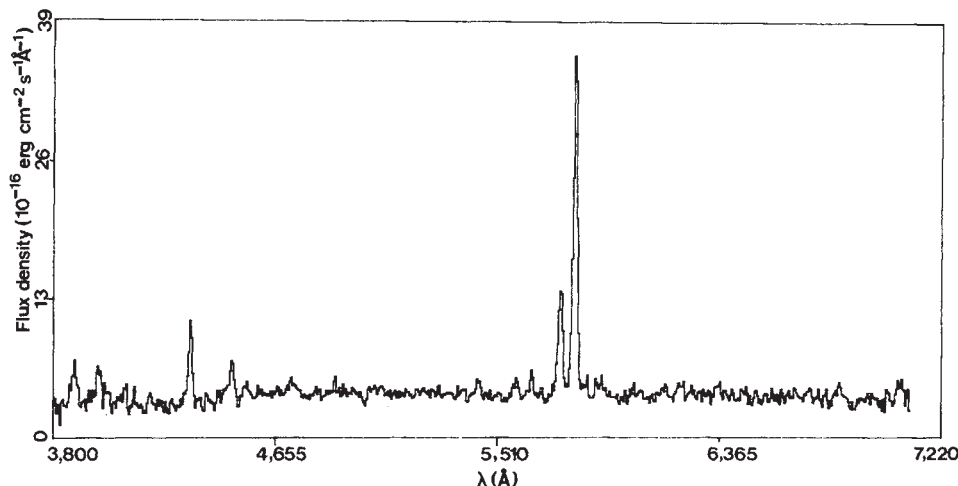


Fig. 2 The spectrum of IRAS1051-273P11 taken with the 3.6-m telescope at La Silla. A correction for the instrumental sensitivity has been applied, which tends to increase the noise on the edges of the spectrum. Note that $H\alpha$ is redshifted out of the wavelength range.

the nucleus, thereby reducing or eliminating the ultraviolet excess required for inclusion in the Markarian survey. Obscuration by dust outside the nucleus could also significantly affect the detectability. Hence the Markarian (ultraviolet) and IRAS (infrared) techniques are complementary indicators of the Seyfert phenomenon.

We show here that an infrared survey can be a highly efficient means of discovering new Seyferts. The nature of the objects selected in this way and their relationship with other active galaxies will be studied further. Comparison of the infrared properties with the intensities and profiles of the narrow emission lines will give unique information about the role of dust in the narrow line region.

A study of the visible morphology and the radio properties

of the infrared Seyferts is of particular interest. These Seyferts may be a link between optically-selected Seyferts, which are associated predominantly with spirals, and the radio-selected ones, which are in elliptical galaxies.

Finally, in addition to the all-sky survey discussed here, a deeper survey of limited regions of the sky was carried out by IRAS. This survey should reach factors of 5-15 times deeper than the all-sky survey and contain several thousand sources and should enable infrared Seyferts to be detected out to redshifts of >0.5 where the effects of cosmological evolution are believed to play an important role.

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1. Stocke, J. T. *et al. Astrophys. J.* **273**, 458-477 (1983).
2. Gioia, I. M. *et al. Astrophys. J.* **283**, 495-511 (1984).
3. Neugebauer, G. *et al. Astrophys. J. Lett.* **278**, L1-L6 (1984).
4. Soifer, B. T. *et al. Astrophys. J. Lett.* **278**, L71-L74 (1984).
5. Young, E. *et al. Astrophys. J. Lett.* **278**, L75-L78 (1984).
6. Habing, H. J. *et al. Astrophys. J. Lett.* **278**, L59-L62 (1984).
7. De Jong, T. *et al. Astrophys. J. Lett.* **278**, L67-L70 (1984).
8. Rieke, G. H. & Low, F. J. *Astrophys. J. Lett.* **199**, L13-L17 (1975).
9. Simon, T. & Dyck, H. M. *Mon. Not. R. astr. Soc.* **172**, 19P-21P (1975).
10. Jameson, R. F., Longmore, A. J., McLinn, J. A. & Woolf, N. J. *Astrophys. J. Lett.* **187**, L109 (1974).
11. Jones, T. W., Leung, C. M., Gould, R. J. & Stein, W. A. *Astrophys. J.* **212**, 51-59 (1977).

12. Miley, G. K. *et al. Astrophys. J. Lett.* **278**, L79-L81 (1984).
13. Neugebauer, G. *et al. Astrophys. J. Lett.* **278**, L83-L85 (1984).
14. *IRAS Circ. 11 Astr. Astrophys.* **134**, C5 (1984).
15. Baldwin, J. A., Phillips, M. M. & Terlevich, R. *Publ. astr. Soc. Pacif.* **93**, 5-19 (1981).
16. Feldmann, F. R., Weedman, D. W., Balzano, V. A. & Ramsey, L. W. *Astrophys. J.* **256**, 427-434 (1982).
17. Véron, M. P. *Astr. Astrophys.* **100**, 12-19 (1981).
18. Shuder, J. M. & Osterbrock, D. E. *Astrophys. J.* **250**, 55-65 (1981).
19. Heckman, T. M., Miley, G. K. & Green, R. F. *Astrophys. J.* **281**, 525-534 (1984).
20. *IRAS Circ. 16 Astr. Astrophys.* (in the press).
21. *IRAS Circ. 6 Astr. Astrophys.* **131**, C1 (1984).
22. Beichman, C. *et al. Astrophys. J. Lett.* (in the press).
23. Véron-Cetty, M. P. & Véron, P. *European Southern Observatory Sci. Rep.* **1**, (1984).
24. Meurs, E. J. A. & Wilson, A. S. *Astr. Astrophys.* (in the press).

On the binary nature of cosmic γ -ray burst sources

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The basic nature of cosmic γ -ray burst sources¹ has remained a mystery. Schaefer² and Schaefer *et al.*³ recently discovered three images on archival photographic plates probably due to optical flashes from the error boxes of three γ -ray burst sources. This discovery opens up the possibility of ground-based optical studies of these objects (see, for example, ref. 4) and provides an important new set of data that can constrain models significantly (see, for example, ref. 5 and references therein) for these intriguing sources. London and Cominsky⁶ have considered a model for the optical flashes, wherein γ -ray bursts are emitted by a collapsed object in a close-binary stellar system and a small fraction of the γ radiation is reprocessed into optical radiation in the surface layers of the

companion star (see refs 7 and 8 for possible alternative explanations). Based on their own estimates of the time, τ , required to reprocess the γ radiation into optical light, London and Cominsky concluded that a binary model would not account for the observed optical flashes. They considered, however, only main-sequence stars and hydrogen-depleted degenerate dwarfs as possible companion stars. We reconsider here the binary model and conclude that it is viable. In particular, under the assumption that the optical flashes were produced by γ -ray bursts of about the same intensity as those observed, we find that nearby (<100 pc) binary systems with secondaries whose masses are less than $\sim 0.06 M_{\odot}$ can fit all the observational constraints for the three optical/ γ -ray pair events (hereafter 1928/1978, 1944/1979 and 1901/1979; see Table 1).

We have considered a wide range of parameters for the binary systems in which the optical flashes may be produced. Here we have chosen specifically a sequence of models that could plausibly represent the evolution of a compact binary during the phase when the mass of the secondary is reduced (by mass transfer) from $\sim 1 M_{\odot}$ to almost planetary values. We adapted the results from a binary evolutionary sequence calculated by Rappaport, Verbunt and Joss⁹, in which the initial binary consists of a collapsed star of mass $1.2 M_{\odot}$ orbiting a $1.0 M_{\odot}$

main-sequence dwarf; the early evolution is dominated by the loss of angular momentum due to magnetic braking, whereas the later evolution is dominated by the emission of gravitational radiation (see model CMB 4 in ref. 9 for details). For the present work, we took the final mass of the primary to be no greater than $1.4 M_{\odot}$ and adjusted the orbital separation accordingly. The evolution of the mass, radius and effective temperature of the companion star in this evolutionary sequence, as well as the orbital period and mass transfer rate, is shown in Fig. 1. As the mass of the secondary, M_c , decreases from $1.0 M_{\odot}$ to $0.1 M_{\odot}$, its properties remain close to those of a main-sequence star of the same mass. For $0.03 \leq M_c \leq 0.1 M_{\odot}$, the secondary star is out of thermal equilibrium and evolving toward a degenerate state; for $M_c \leq 0.03 M_{\odot}$, the internal properties of the secondary are essentially those of a completely degenerate star. The results described below are not especially sensitive to the details of this evolutionary scenario; the calculated optical fluences depend primarily on the current orbital separation and the radius and effective temperature of the secondary.

We do not attempt here to model the γ -ray burst emission mechanism, but assume merely that γ radiation is emitted isotropically by the collapsed star. For each of the binary models, we consider γ -ray burst energies, E_{γ} , in the range of 10^{35} – 10^{39} erg and use an approximate expression for the average γ -ray fluence, F_{γ} , that is absorbed over the facing hemisphere of the companion star

$$F_{\gamma} = \frac{E_{\gamma}(1-\alpha)\{1-[1-(R_c/a)^2]^{1/2}\}}{4\pi R_c^2} \\ \approx \frac{E_{\gamma}}{8\pi a^2} \approx 1.6 \times 10^{14} \left[\frac{E_{\gamma}}{10^{37} \text{ erg}} \right] \\ \times \left[\frac{a}{5 \times 10^{10} \text{ cm}} \right]^{-2} \text{ erg cm}^{-2} \quad (1)$$

where α is the γ -ray albedo (taken to be 0.2), R_c is the radius of the secondary and a is the orbital separation.

The γ radiation, which penetrates the surface layers of the companion star, is thermalized and re-radiated as optical light (and possibly also as infrared and ultraviolet radiation). We have carried out Monte Carlo calculations to determine the column density beneath the stellar surface at which the burst energy is deposited and find that for typical burst spectra, the amount of energy deposited falls off approximately exponentially, with an e-folding column density of ~ 8 – 10 g cm^{-2} (see also ref. 6).

London and Cominsky⁶ have estimated the reprocessing time, τ , and find that for γ -ray fluences (at the companion) in the range $10^{12.4} < F_{\gamma} < 10^{14.8} \text{ erg cm}^{-2}$, the value of τ can greatly exceed the duration of the γ -ray burst. Their estimates, however, are only of order of magnitude accuracy and neglect the effects of the hydrodynamic expansion of the envelope of the secondary. Therefore, we consider here a rather wide range of reprocessing times of 5–500 s. The lower limit is set by the typical duration of the γ -ray burst events^{10,11}, whereas the upper limit is set by the lack of trailing of two of the detected optical images^{2,3} (see Table 1). For simplicity, we assume that during the optical flash the heated hemisphere of the companion star radiates uniformly for a time τ at a constant temperature, $T_b = [F_{\gamma}/\sigma\tau + T_{\text{qui}}^4]^{1/4}$ (see also ref. 6), where σ is the Stefan-Boltzmann constant and T_{qui} is the effective temperature of the companion star in quiescence between γ -ray burst events.

We calculated the optical fluence at the Earth in a 1,000-Å-wide band centred at 4,350 Å (a blue band that approximates the response of the archival plates on which the optical flashes were discovered^{2,3}) for each model in our evolutionary sequence and for each of several X-ray burst energies within the range prescribed above. For simplicity, we assumed a typical aspect angle of 45° between the line of sight and the line connecting the centres of the binary components. We also computed the distance to the source implied by the measured γ -ray fluence of each particular event at the Earth (see Table 1) and the

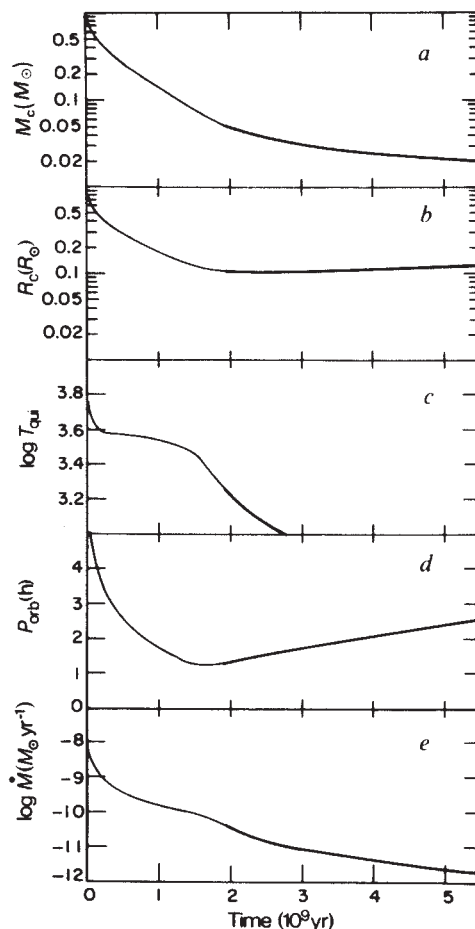


Fig. 1 Evolution of a compact binary system under the influence of magnetic braking and gravitational radiation (adapted from model CMB4 of ref. 9). The initial binary system consists of a $1.0 M_{\odot}$ main-sequence dwarf companion and a $1.2 M_{\odot}$ collapsed star in a 6.3-h orbit. The quantities plotted as functions of elapsed time in frames a–e are the mass, radius and quiescent effective temperature of the secondary star, the orbital period and the rate of mass loss from the secondary, respectively. The heavy portion of each curve corresponds to binary models which yield optical flashes (due to the reprocessing of γ -radiation) with properties similar to those observed^{2,3}. The heavy portion of the effective temperature curve is qualitatively correct, but its quantitative details are highly uncertain.

assumed γ -ray burst energy, and the quiescent visual magnitude m_{qui} of the companion star. We require that $m_{\text{qui}} > 24$ for a model to be consistent with the lack of any prominent optical candidates^{12–15} in the error boxes of the three γ -ray burst sources that have been associated with optical flashes^{2,3}.

We show sample results of our calculations in Fig. 2 for the 1928/1978 source (results for the 1944/1979 and 1901/1979 sources being similar). The γ -ray fluence of the 1978 event was $\sim 3 \times 10^{-4} \text{ erg cm}^{-2} \text{ s}^{-1}$, whereas the blue magnitude of the optical flash on the archival plate was 10.2 (ref. 2). This corresponds to a blue fluence, F_{opt} , of $\sim 1.4 \times 10^{-6} \mathcal{R} \text{ erg cm}^{-2}$, where $\mathcal{R}$ is a correction factor that takes into account the reciprocity failure¹⁶ of the photographic emulsion. If the entire optical event transpired during the exposure of the plate, we obtain (from data presented in ref. 16) $\mathcal{R} = (\tau/T_{\text{exp}})^{0.18}$, where T_{exp} is the exposure time of the plate, is 2,700 s for the plate on which the 1928 optical flash was discovered.

The results in Fig. 2 show the predicted values of F_{opt} as a function of the assumed γ -ray burst energy. Each burst energy corresponds to a unique distance to the source that generated the 19 November 1978 event if the model is to account for the measured γ -ray fluence. We show the results for binary models with several different values of M_c and $\tau_{\odot}$. (Our estimates for

Table 1 Properties of optical flashes from γ -ray burst sources*

Event pair name	1928/1978	1944/1979	1901/1979
γ -Ray burst source	19 Nov. 1978 GRB	13 Jan. 1979 GRB	5 Nov. 1979 GRB
γ -Ray fluence (erg cm^{-2}) $\ddagger$	3.2×10^{-4}	1.1×10^{-4}	1.3×10^{-5}
Depth of optical flash	17 Nov. 1928	19 Feb. 1944	4 Oct. 1901
Blue magnitude of flash	10.2	12.1	12.6 $\dagger$
Optical fluence (erg cm^{-2}) $\S$	4.7×10^{-7}	1.4×10^{-7}	1.6×10^{-8}
Plate exposure (s)	2,700	5,400	660
Trailing limit (s) $\parallel$	600	—	200

* The data are taken from refs 2 and 3.

$\dagger$ See ref. 16.

$\ddagger$ For $E \geq 30$ keV

$\S$ In a 1,000-Å band centred at 4,350 Å, for an assumed value of $\tau = 5$ s.

$\parallel$ Limit on the duration of the optical flash derived from the lack of trailing of the observed images.

F_{opt} are effectively lower limits, as the presence of a disk or ring of matter in the binary stellar system would probably increase the total blue fluence resulting from reprocessing. The measured value of F_{opt} , which varies by up to a factor of two for different choices of τ because of the variation of the reciprocity correction, is also indicated.

We consider the agreement between the model calculations and the observations to be excellent when the following sources of error are taken into account: (1) the uncertainties in establishing the apparent magnitude of the optical flash (including uncertainties in reciprocity corrections for old emulsions¹⁶ and uncertainties in comparing the untrailing flash image with trailed reference-star images¹⁶); (2) the uncertainty in the measured γ -ray fluence; (3) the unknown difference between the strength of the γ -ray burst that produced the 1928 optical flash and the observed γ -ray burst of 1978. In the light of these uncertainties, we consider agreement to within a factor of ~ 5 between the predicted and measured optical fluences to be an acceptable fit (see Fig. 2). In fact, as is evident from Fig. 2, there are a number of models with $M_c \leq 0.05 M_\odot$ for which the predicted optical fluence is within a factor of ~ 2 –3 of the measured fluence. Binary models providing acceptable fits are shown as heavy curves in Fig. 1. For each value of τ and M_c , the best-fit model roughly corresponds to the γ -ray energy that yields $T_b \approx 8,500$ K and thereby maximizes the amount of reprocessed radiation in the blue band.

From the results shown in Fig. 2, we conclude that the proposed model for producing the optical flashes by reprocessing γ radiation on the surface of a binary stellar companion is viable. London and Cominsky⁶ reached the opposite conclusion because they did not fully consider extremely-low-mass hydrogen-rich secondary stars. We find that the most favourable models for the 1928/1978 event are those with $M_c \leq 0.05 M_\odot$, $T_{\text{qui}} \leq 1,800$ K, $E_\gamma \leq 10^{38}$ erg and distances $d \leq 50$ pc. Values of E_γ and d less than $\sim 10^{35}$ erg and ~ 2 pc, respectively, are allowed by the binary model, but are excluded by considerations (discussed below) that are based on the Oort limit and the diffuse cosmic X-ray background.

The same type of calculation as that represented in Fig. 2 was repeated for the 1944/1979 and 1901/1979 events. The binary models best able to account for these events are essentially the same as for the 1928/1978 event (Fig. 1). Acceptable distances are ≤ 100 and ≤ 250 pc for the 1944/1979 and 1901/1979 events, respectively.

Distances for these sources of ≤ 2 pc are excluded because the mass density of such systems would then exceed the Oort limit on the local density of matter in the solar neighbourhood ($\sim 0.15 M_\odot \text{ pc}^{-3}$; see, for example, refs 12, 17 and 18). Moreover, if (1) the mean distance between sources is ≤ 10 pc, (2) the systems have neutron-star primaries, (3) these systems were low-mass X-ray binaries at an earlier evolutionary phase¹⁹ and if (4) the number of such systems in our Galaxy is typical of

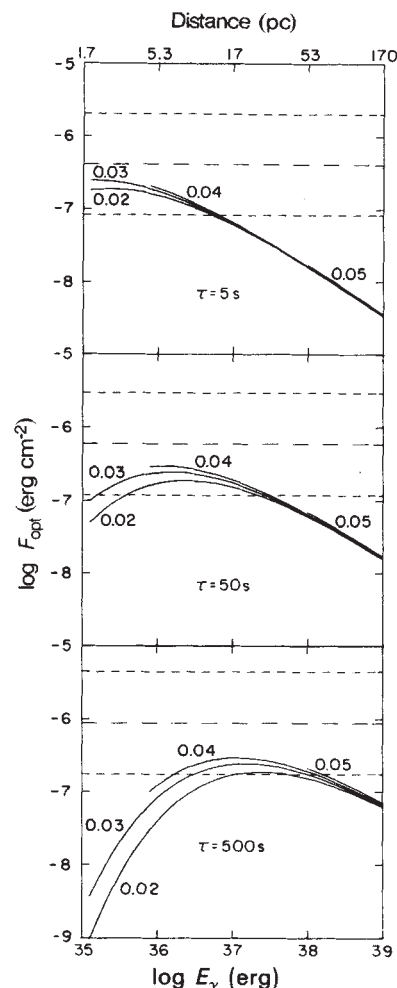


Fig. 2 Calculated optical fluences, F_{opt} , at the Earth, in a 1,000-Å band centred at 4,350 Å. The calculations are carried out for a γ -ray fluence at the Earth of $3 \times 10^{-4} \text{ erg cm}^{-2}$ and for a range of binary models, assumed γ -ray burst energies, E_γ , and reprocessing times, τ . The binary model is completely specified by the mass of the secondary, M_c (see Fig. 1); sample results are shown for $M_c = 0.02, 0.03, 0.04$ and $0.05 M_\odot$. The curve for each value of M_c terminates at the point where the companion star would be visible in quiescence ($m_{\text{qui}} < 24$; see text). The long dashed line in each frame is the measured optical fluence² of the 1928/1978 event (see Table 1). Note that this quantity attains a somewhat different value for each of the assumed reprocessing times because of variations in the correction for reciprocity failure. The set of short dashed curves in each frame indicates a range of a factor of 5 around the measured optical fluence, within which the model fits are considered to be acceptable.

other galaxies, then the total X-ray emission from such systems in distant galaxies would dominate the diffuse X-ray background²⁰.

The need for more detailed calculations of the γ -ray reprocessing times is evident from Fig. 2. If we knew τ more reliably (for a given model of the secondary star and a given γ -ray fluence at the secondary), a much more restrictive range of distances to each source could be obtained.

If the reprocessing model is the correct explanation of the optical flashes from γ -ray burst sources, we have already established some very specific properties of these sources. They are binaries containing a low-mass cool secondary star, in orbit with a collapsed star (most probably a neutron star or degenerate dwarf) that is the source of the γ -ray bursts. The particular systems that produced the observed optical flashes may be relatively close neighbours of the Solar System; this inference is consistent with the unusually large measured γ -ray fluences^{10,11} of the 1928/1978 and 1944/1979 events.

The type of binary system we have invoked, although yielding apparently correct γ -ray reprocessing properties, presents a difficulty that must be resolved if the model is to be viable. The mass-loss rates from the secondary lie in the range of $\sim 0.2\text{--}3 \times 10^{-11} M_{\odot} \text{ yr}^{-1}$ (Fig. 1). If this matter were accreted onto a $\sim 1 M_{\odot}$ collapsed star and emitted isotropically in the X-ray range, the flux at the Earth would be

$$F_x \approx 7 \times 10^9 \left[\frac{\dot{M}}{10^{-12} M_{\odot} \text{ yr}^{-1}} \right] \left[\frac{D}{100 \text{ pc}} \right]^{-2} \times \left[\frac{R}{10^6 \text{ cm}} \right]^{-1} \text{ erg cm}^{-2} \text{ s}^{-1} \quad (2)$$

where R is the radius of the collapsed star. The measured X-ray flux from the 19 November 1978 source in quiescence is $\sim 10^{-13} \text{ erg cm}^{-2} \text{ s}^{-1}$ (ref. 21), which is ~ 5 orders of magnitude below the level implied by equation (2) for a neutron star, at a distance of $\leq 100 \text{ pc}$, accreting at $\geq 10^{-12} M_{\odot} \text{ yr}^{-1}$. This dilemma may be resolved if there is a reduction or cessation in the rate at which matter is steadily accreted onto the collapsed star when the mass-loss rate from the secondary falls below some critical value. (We note that this picture may support some theoretical ideas concerning the nature of γ -ray bursts²².) It is also possible that the accretion-driven luminosity will be emitted at ultraviolet or very soft X-ray energies when the accretion rate is very low.

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- Kiebasdel, R. W., Strong, I. B. & Olson, R. A. *Astrophys. J. Lett.* **182**, L85–L88 (1973).
- Schaefer, B. E. *Nature* **294**, 722–724 (1981).
- Schaefer, B. E. *et al. Astrophys. J. Lett.* **286**, L1–L4 (1984).
- Ricker, G. R., Doty, J. P., Vallerger, J. V. & Vanderspek, R. K. *Soc. Photo-Opt. Instrum. Engr.* **445**, 370 (1984).
- High-Energy Transients in Astrophysics* (ed. Woosley, S. E.) (American Institute of Physics, New York, 1984).
- London, R. A. & Cominsky, L. R. *Astrophys. J. Lett.* **275**, L59–L63 (1983).
- Woosley, S. E. in *High-Energy Transients in Astrophysics* (ed. Woosley, S. E.) 485 (American Institute of Physics, New York, 1984).
- Katz, J. *Astrophys. Lett.* (in the press).
- Rappaport, S., Verbunt, F. & Joss, P. C. *Astrophys. J.* **275**, 713–731 (1983).
- Mazets, E. P. *et al. Astrophys. Space Sci.* **80**, 3–83 (1981).
- Mazets, E. P. *et al. Astrophys. Space Sci.* **80**, 119–143 (1981).
- Schaefer, B. E. & Ricker, G. R. *Nature* **302**, 43–45 (1983).
- Schaefer, B. E., Seitzer, P. & Bradt, H. V. D. *Astrophys. J. Lett.* **270**, L49–L52 (1983).
- Pedersen, H. *et al. Astrophys. J. Lett.* **270**, L43–L47 (1983).
- Barat, C. *et al. Astrophys. J. Lett.* **286**, L5–L9 (1984).
- Schaefer, B. E. thesis, Massachusetts Inst. Technology (1983).
- Oort, J. H. *Bull. Astr. Inst. Neth.* **15**, 45 (1960).
- Bahcall, J., Hut, P. & Tremaine, S. *Astrophys. J.* (in the press).
- Ventura, J., Bonazzola, S., Hameury, J. M. & Heyvaerts, J. *Nature*, **301**, 491–493 (1983).
- Elvis, M., Soltan, A. & Keel, W. C. *Astrophys. J.* **283**, 479–485 (1984).
- Grindlay, J. E. *et al. Nature* **300**, 730–731 (1982).
- Joss, P. C. & Rappaport, S. in *High-Energy Transients in Astrophysics* (ed. Woosley, S. E.) 555 (American Institute of Physics, New York, 1984).

Theory of radar imaging of internal waves

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Radar images taken over ocean areas, in particular, those obtained by the synthetic aperture radar (SAR) onboard the American Seasat satellite in 1978^{1,2}, sometimes show features that seem to be surface manifestations of oceanic internal waves^{3–11}. Here I present a theory explaining the large radar signatures of internal waves in which the imaging is attributed to variations in the short-scale surface roughness induced by current variations associated with internal waves.

Figure 1 is an example of Seasat SAR image of coastal waters west of Portugal showing surface expressions of nonlinear internal wave trains propagating towards the coast. The internal wave groups are believed to be generated at the continental shelf break by interaction of the tidal current with underwater bottom topography. Within each group the wavelength decreases with distance from the leading crest, indicating that they represent nonlinear dispersive wave trains; their partly irregular structure is attributed to refraction effects caused by the shoaling bottom and to interactions with the current field.

It may seem surprising that internal waves can generate such large signatures in radar imagery. These signatures must originate from the sea surface, as the penetration depth of the microwaves emitted by the radar in the sea water is only a fraction of their wavelength. The radar senses the short-scale roughness of the ocean surface by means of Bragg scattering. Thus, the radar signatures must be the result of a modulation of the short surface waves, generated by interactions with the internal waves¹²; this modulation can either be achieved by surface films (slicks) that accumulate in flow convergence zones and damp the short surface waves there¹³, or by hydrodynamic interaction of these waves with the horizontal surface current associated with internal wave motion^{14–17}.

The first mechanism is often active in coastal waters where surface films, either of natural or anthropogenic origin, are present. These films, even when monomolecular, damp short surface waves very strongly^{18–20}, reducing the radar reflectivity accordingly. When slicks modulate the short-scale surface roughness, the radar image of an internal wave field consists of dark streaks (areas of reduced radar backscatter) on a uniform bright background. In most cases, however, the radar signatures of internal waves have double sign, which means that the corresponding radar image consists of bright and dark streaks associated respectively with enhanced and reduced radar reflectivity as compared with the local mean, indicative of hydrodynamic modulation. Although most investigators now hold that the latter mechanism is responsible for the imaging^{7–9}, the question of why these radar signatures are so large has not been answered satisfactorily. I have attempted to do so here using the weak hydrodynamic interaction theory in the relaxation time approximation, the crucial point being that another approximation is applicable compared with the case of the modulation of short by long surface waves^{21–23}.

Typical imaging radars operate at wavelengths λ_0 in the centimetre to decimetre range and at incidence angles θ of $20\text{--}70^\circ$, within which the radar backscattering is dominated by Bragg scattering^{24–26}. In this case the normalized radar backscattering cross-section, σ , is proportional to the spectral energy density, E , of the Bragg waves, which have a wavelength of $\lambda = \lambda_0/2 \sin \theta$ and which travel towards or away from the antenna look-direction

$$\sigma = T(E(+2k_0) + E(-2k_0)) \quad (1)$$

where k_0 denotes the projection of the radar wave vector on the horizontal plane and T a scattering coefficient which can be calculated from Bragg scattering theory and which depends on incidence angle, dielectric constant, radar wavelength and polarization (see, for example, refs 24, 26). The synthetic aperture radar on Seasat had a wavelength of $\lambda_0 = 0.235 \text{ m}$ (L-band) and operated at incidence angles of $\sim 23^\circ$; the short waves responsible for the backscattering had a wavelength of 34 cm and a wave period of 0.47 s .

Equation (1) relates the modulation of the normalized radar cross-section to that of the spectral energy density $E(\pm 2k_0)$ of the Bragg waves, which reduces the problem to that of the modulation of the Bragg waves by the surface current associated with internal waves.

Typically, internal waves visible on radar images have wavelengths greater than several hundred metres and wave periods $> 10 \text{ min}$; the space and time scales of the currents associated with these waves are much greater than those of the

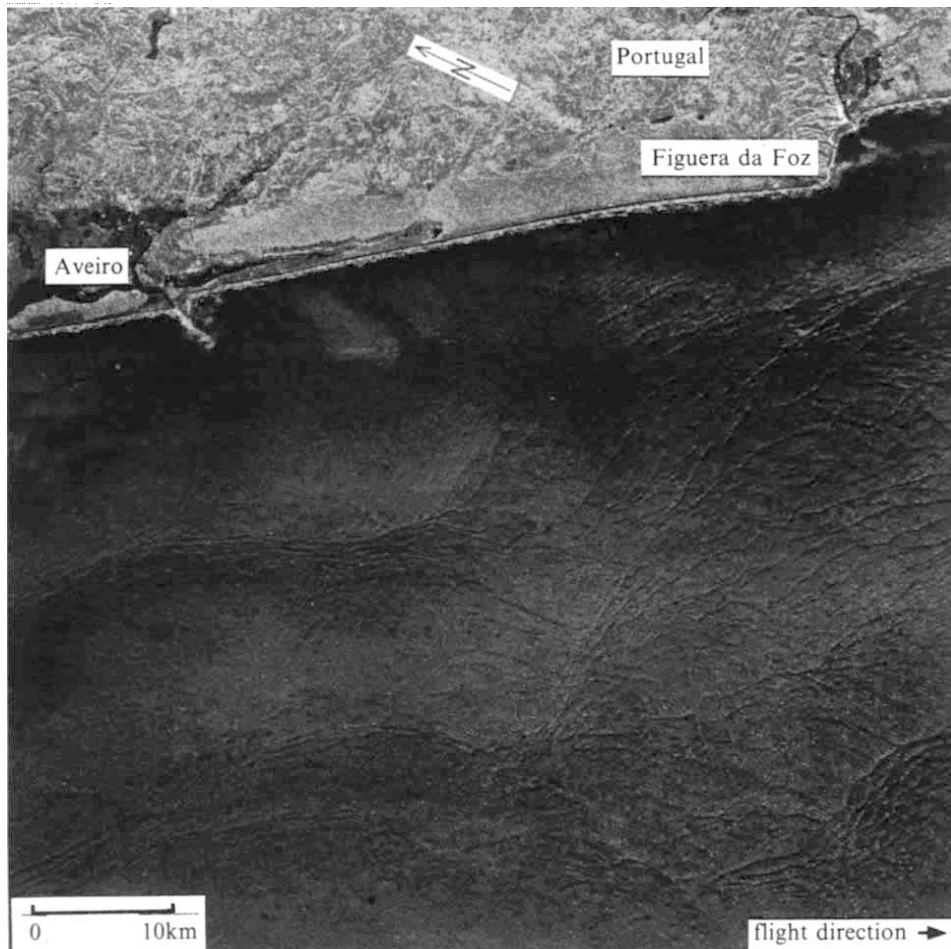


Fig. 1 Synthetic aperture radar image of Atlantic coastal waters off the Portuguese coast near Figueira da Foz and Aveiro. This radar image was taken from the Seasat satellite on 20 August 1978, 21:42 UT (orbit 785). It shows surface manifestations of a large number of nonlinear internal wave trains generated by the action of the tide at the continental shelf break.

Bragg waves and therefore we can apply a Wentzel-Kramers-Brillouin-type theory to describe their interaction. In this theory the transport equation describing the variation of the spectral energy density of short waves in a slowly varying current field is the action balance or radiation balance equation^{21-23,27}

$$\frac{dN}{dt} = \left(\frac{\partial}{\partial t} + \dot{x} \cdot \frac{\partial}{\partial x} + \dot{k} \cdot \frac{\partial}{\partial k} \right) N = S(x, k, t) \quad (2)$$

where $N(x, k, t) = E(x, k, t)/\omega'$ is the action spectrum, $E(x, k, t)$ the wave spectrum, ω' the intrinsic frequency of the wave in a reference system which is locally at rest, x the space variable, k the wavenumber and $S(x, k, t)$ a source function. The waves propagate along trajectories in four-dimensional phase space, given by the ray equations, $\dot{x} = \partial\omega/\partial k$ and $\dot{k} = -\partial\omega/\partial x$, where $\omega(x, k, t) = \omega'(k) + k \cdot U(x, t)$ denotes the wave frequency in the moving medium with variable velocity $U(x, t)$.

We assume that the variable surface current leads to only small deviations of the action density from equilibrium and therefore write the action density, N , and the surface current, U , as sums of a constant equilibrium term and a time-dependent perturbation term, $N(x, k, t) = N_0(k) + \delta N(x, k, t)$ and $U(x, t) = U_0 + \delta U(x, t)$. Furthermore, for small perturbations we can approximate the source term S by a diagonal operator, $S(x, k, t) = -\mu \delta N(x, k, t)$, where μ is a parameter with dimension (time)⁻¹, called relaxation rate, and $\tau_r = \mu^{-1}$ is the relaxation time, a system constant determined by the combined effects of wind excitation, energy transfer to other waves caused by conservative resonant wave-wave interaction and energy loss through dissipative processes such as wave breaking. No direct measurements of τ_r in the open ocean exist, but theory predicts that it is of the order of 10–100 wave periods. Applied to Seasat-SAR Bragg waves, this means that τ_r should lie in the range 4.7–47 s. Inserting the above equation for N , U , S and

$\dot{k}$ into equation (2) and retaining only first-order terms gives

$$\left(\frac{\partial}{\partial t} + (c_g + U_0) \cdot \frac{\partial}{\partial x} + \mu \right) \delta N = k \cdot \frac{\partial U}{\partial x} \cdot \frac{\partial N_0}{\partial k} \quad (3)$$

where $c_g = \partial\omega'/\partial k$ is the group velocity. The time scales of the three terms on the left-hand side are the local time, τ , the advection time, τ_a , and the relaxation time, τ_r ; $\tau = \Omega^{-1}$, where Ω is the radian frequency of the internal waves, and $\tau_a = |(c_g + U_0) \cdot K|^{-1}$, where K denotes the wave vector of the internal wave. In general, both τ and τ_a are $\gg 1$ min; consequently, the first two terms on the left-hand side of equation (3) should be much smaller than the third, the relaxation term, which alone is retained.

Specializing equation (3) to describe the modulation of Bragg waves, defining the projection of the antenna axis on the horizontal plane as x axis, we obtain

$$\frac{\delta\sigma}{\sigma_0} = \frac{\delta N}{N_0} = \frac{\delta E}{E_0} = -(4 + \gamma)\tau_r \frac{\partial U_x}{\partial x} \quad (4)$$

Here $\delta\sigma = \sigma - \sigma_0$ denotes the deviation of the normalized radar cross-section, σ , from its mean value σ_0 ; γ is the ratio between the group and phase velocity of the Bragg waves ($\gamma = 0.5$ for gravity waves). In deriving equation (4) we have assumed that $E \propto |k|^{-4}$, that is, E has the form of the Phillips equilibrium spectrum¹⁵, and that σ is related to E through equation (1). Equation (4) is our principal result here, showing that the cross-section modulation caused by internal waves is proportional to the product of the surface current gradient in the look-direction of the antenna and the relaxation time, τ_r .

An analysis of Seasat-SAR imagery of underwater sandbanks in the Southern Bight of the North Sea²⁷ has estimated the relaxation time of the Bragg waves; for a wind speed of 4 m s⁻¹,

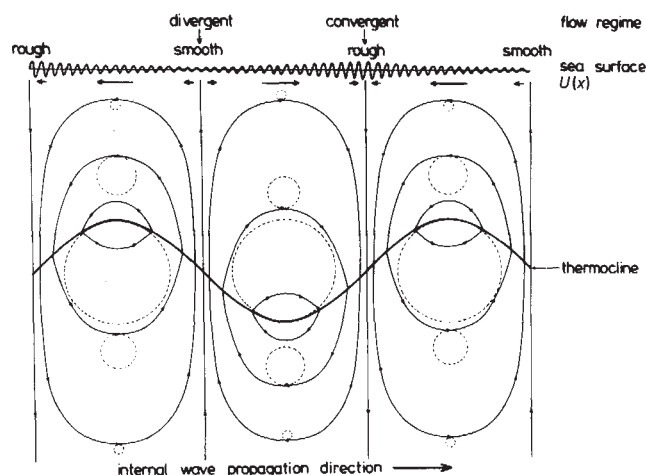


Fig. 2 Schematic plot of streamlines and particle velocities associated with a linear internal wave propagating along a sharp thermocline (adopted from ref. 28). The short-scale surface roughness is modulated by the surface current associated with the internal waves.

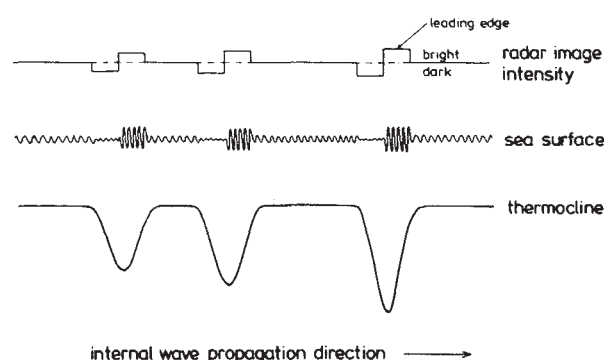


Fig. 3 Schematic plot of the thermocline displacement, the surface roughness modulation and the radar image intensity modulation associated with a nonlinear internal wave packet travelling along a shallow thermocline. The leading edge of the wave packet is associated with increased surface roughness leading to a bright band in the radar image.

it is ~ 30 – 40 s, corresponding to 60–80 wave periods. This value of τ_r seems large, but is still within the predicted range.

Note that if we wanted to describe the cross-section modulation by long surface waves, equation (4) would not be applicable. In this case the corresponding equation would be

$$\frac{\delta\sigma}{\delta_0} = \frac{\delta N}{N_0} = \frac{\delta E}{E_0} = -\left(\frac{4+\gamma}{\hat{\omega}}\right) \frac{\partial U_x}{\partial x} \quad (5)$$

where $\hat{\omega}$ denotes the radian frequency of the long surface wave and U the associated orbital velocity. Note that in equation (5) the factor in parenthesis is typically a factor of 10–20 larger than the corresponding factor in equation (4). The other factor in equation (4), the horizontal current gradient (or strain rate), $\partial U_x/\partial x$, can be calculated from internal wave theory¹⁵.

Figure 2 shows the streamlines and water-particle orbits in a linear internal wave travelling from left to right at the boundary of two water layers of different density (thermocline). The internal waves cause almost no elevation of the surface and are associated with a spatially and temporally varying surface current field which interacts with the surface waves, giving rise to the surface-roughness modulation depicted in Fig. 2.

In the case of a nonlinear internal wave packet travelling from left to right, the vertical displacement of the thermocline and the roughness pattern have qualitatively the form shown in Fig. 3. The roughness pattern consists of pairs of adjacent very rough and smooth streaks separated by broad areas with a mean local roughness; the associated radar image therefore consists of pairs of adjacent bright and dark bands on a uniform background.

The leading edge of the nonlinear internal wave train is associated with a convergence zone²⁹, giving rise to a bright band in the radar image.

Typical values for the strain rate of linear internal waves in coastal regions are 10^{-3} s^{-1} (ref. 7), but can be more than an order of magnitude greater for strongly nonlinear waves. If a surface current gradient of 10^{-3} s^{-1} and a relaxation time of $\tau_r = \mu^{-1} = 40 \text{ s}$ is inserted into equation (4), then one obtains for the modulation depth, $\delta\sigma/\sigma_0 \sim 0.2$, values commonly observed in radar imagery of internal waves. Although the surface current gradient associated with internal waves is typically an order of magnitude smaller than that associated with long surface waves, the modulation depth of internal waves can nevertheless be stronger than that of long surface waves, because the factor by which the surface current gradient is multiplied is an order of magnitude greater for internal waves (compare equations (4) and (5)).

The physical explanation for the large cross-section modulations obtained with our theory is as follows. In the case of internal waves, the straining exerted on the short-wave system by the current gradient is balanced by the relaxation of the wave system. This balance is different from the one active in the modulation of short waves by long surface waves, where the straining is balanced mainly by the temporal variation of the orbital velocity. Typically, the time scale of the relaxation (the relaxation time) is an order of magnitude greater than that of the orbital velocity, given by $\hat{\omega}^{-1}$. Thus in the case of short-wave modulation by internal waves, the straining can act sufficiently long on the short-wave system for a strong modulation to build up. Indeed the modulation is only limited by the relaxation time, which is a parameter describing how much disequilibrium the short-wave system can endure while subject to strain by a variable current.

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- Born, G. H., Dunne, J. A. & Lame, D. B. *Science* **204**, 1405–1406 (1979).
- Jordan, R. L. *IEEE J. Oceanic Engng* OE-5, 154–164 (1980).
- Elachi, C. & Apel, J. R. *Geophys. Res. Lett.* **3**, 647–650 (1976).
- Apel, J. R. in *Oceanography from Space* (ed. Gower, J. F. R. 525–533 (Plenum, New York, 1981).
- Vesceky, J. F. & Stewart, R. H. *J. geophys. Res.* **87**, 3397–3430 (1982).
- Trask, R. P. & Briscoe, M. G. *J. geophys. Res.* **88**, 1789–1799 (1983).
- Alpers, W. & Salusti, E. *J. geophys. Res.* **88**, 1800–1808 (1983).
- Hughes, B. A. & Gower, J. F. R. *J. geophys. Res.* **88**, 1809–1824 (1983).
- Apel, J. R. & Gonzales, F. I. *J. geophys. Res.* **88**, 4459–4466 (1983).
- Fu, L.-L. & Holt, B. *J. geophys. Res.* **89**, 2053–2060 (1984).
- Bagg, M. T. & Johnson, K. I. Admiralty Research Establishment, Portland (UK) Tech. Note 720/84 (1984).
- Brown, W. E. Jr, Elachi, C. & Thompson, T. W. *J. geophys. Res.* **81**, 2657–2667 (1976).
- Lafond, E. C. *The Sea Vol. 1* (ed. Hill, M. N.) 731–751 (Interscience, New York, 1962).

- Phillips, O. M. *Phys. Atmos. Oceans* **9**, 954–961 (1973).
- Phillips, O. M. *The Dynamics of the Upper Ocean*, 199–255 (Cambridge University Press, 1977).
- Hughes, B. A. *J. geophys. Res.* **83**, 455–465 (1978).
- Gargett, A. E. & Hughes, B. A. *J. Fluid Mech.* **52**, 179–192 (1981).
- Huehnerrfuss, H., Alpers, W. & Jones, W. L. *Radio Sci.* **13**, 979–983 (1978).
- Huehnerrfuss, H., Alpers, W., Jones, W. L., Lange, P. A. & Richter, K. *J. geophys. Res.* **86**, 429–438 (1981).
- Huehnerrfuss, H. et al. *J. geophys. Res.* **88**, 9817–9822 (1983).
- Keller, W. C. & Wright, J. W. *Radio Sci.* **10**, 139–147 (1975).
- Alpers, W. & Hasselmann, K. *Boundary-Layer Met.* **13**, 215–230 (1978).
- Alpers, W., Ross, D. B. & Rufenach, C. L. *J. geophys. Res.* **86**, 6481–6498 (1981).
- Wright, J. W. *IEEE Trans. Antennas Propagation* AP-16, 217–223 (1968).
- Wright, J. W. *Boundary-Layer Met.* **13**, 87–105 (1978).
- Valenzuela, C. R. *Boundary-Layer Met.* **13**, 61–85 (1978).
- Alpers, W. & Hennings, I. *J. geophys. Res.* **89**, 10529–10546 (1984).
- Defant, A. *Physical Oceanography Vol. 2*, 517–570 (Pergamon, Oxford, 1961).
- Osborne, A. R. & Burch, T. L. *Science* **208**, 451–460 (1980).

Martian atmospheric carbon dioxide and weathering products in SNC meteorites

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SNC meteorites—four shergottites, three nakhlites and Chassigny—are postulated to have originated on Mars¹. Their late crystallization ages (<1,300 Myr compared with 4,600 Myr for other igneous meteorites) and the presence, in shock-produced glass in EETA79001, of noble gas² and nitrogen³ components resembling the martian atmosphere provide evidence for such a provenance. If this interpretation is correct then carbon dioxide, by far the most abundant constituent of the martian atmosphere⁴, should also be present. The stepped combustions described here show that most of the carbon present in the samples can be ascribed to terrestrial contamination. EETA79001, however, contains 4.6 p.p.m. of an isotopically distinct component enriched in ¹³C ($\delta^{13}\text{C} = +36\%$), whereas high-temperature carbon of inferred igneous origin in this meteorite and other SNCs has a $\delta^{13}\text{C}$ value of about -30% . The ¹²C/¹³C ratio of the isotopically heavy component is within the error limits of Viking measurements⁴ in the martian atmosphere and, thus, strengthens the case for a planetary origin. Another carbon-containing species, believed to be carbonate, has been found in Nakhla ($\delta^{13}\text{C} = \text{between } +12 \text{ and } +24\%$) and may be a product of atmospheric weathering on Mars.

Analyses of SNC meteorites were performed using a stepped combustion technique, similar to that of Swart *et al.*⁵; isotope measurements were made using a high-sensitivity static mass spectrometer capable of determining $\delta^{13}\text{C}$ values to a precision of $\pm 3\%$ or better on samples as small as 6×10^{-9} g carbon in the form of carbon dioxide (R.H.C., I.P.W., A. W. Joines and C.T.P., in preparation). ($\delta^{13}\text{C}(\%) = (R_{\text{sam}}/R_{\text{std}} - 1) \times 1,000$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ and $R_{\text{std}} = 0.0112372$.)

Bulk carbon contents and isotope compositions for all the meteorites obtained by summation of data from individual extraction steps are plotted in Fig. 1a. The total amounts of carbon present in the meteorites define three groups: (1) those recovered from the Antarctic ice cap, ignoring for the moment lithology B of EETA79001, have the lowest carbon contents (<150 p.p.m.); (2) Gobernador Valadares and Lafayette (>750 p.p.m. C) were found after an unknown length of time on Earth; (3) samples with intermediate carbon contents are all meteorites observed to fall and recovered soon afterwards. This pattern, which reflects the known contamination hazards^{6,7} of the terrestrial environment, suggests that the carbon content of SNC meteorites is dominated by biological organic matter produced on Earth. Such a conclusion is supported by the bulk $\delta^{13}\text{C}$ data which, with the exception of Nakhla, lie in the range -20 to -30% . Lithology B of EETA79001 was processed in a slightly different way in our laboratory and may have acquired much of its relatively large carbon content as contamination there rather than in the natural environment before collection.

The above data demonstrate the ubiquity of contamination and exemplify the difficulties involved in obtaining useful carbon isotope information from samples with a low indigenous carbon content. The problems of contamination can be overcome to some extent, however, by consideration of the carbon released during high-temperature steps of the stepped combustion. Although most terrestrial organics burn at low temperatures^{5,8}, it is probably not possible to choose a unique temperature for differentiating between contamination and indigenous carbon. We have chosen to consider the carbon which combusts above 600 °C. Figure 1b plots the summed abundance of carbon extracted above 600 °C against isotope composition. Chassigny occupies a unique position in the plot. The shergottites, with the exception of one sample of Shergotty, and the nakhlites, which are closely grouped, appear to define a two-component mixing trend, with one component an isotopically heavy species

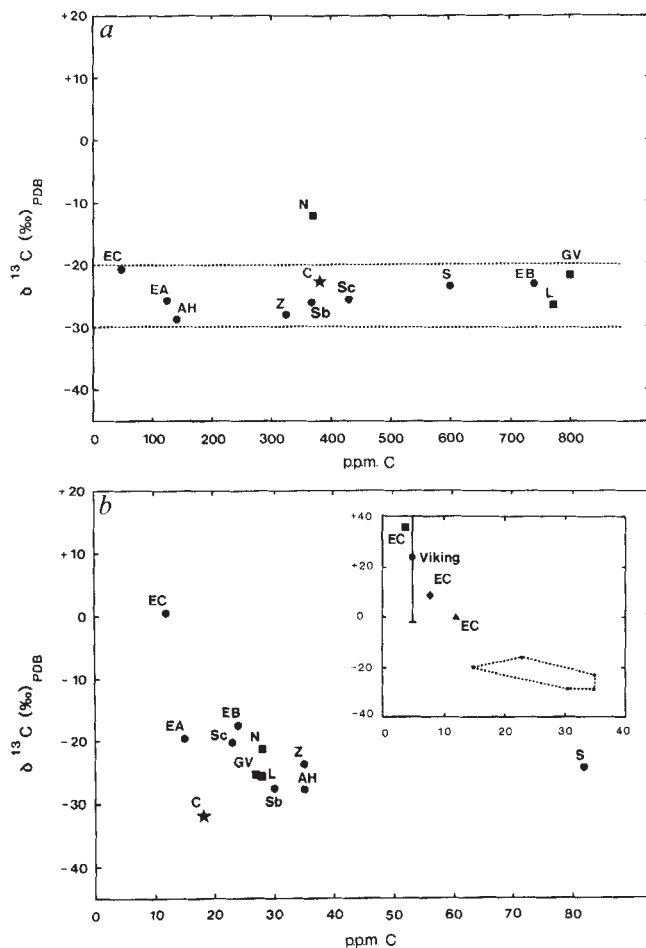


Fig. 1 a, Calculated bulk carbon contents and isotope compositions for samples of the shergottites (●), the nakhlites (■) and the chassignite (★). b, Data from >600 °C extraction steps. Inset, comparison of the SNC dataset with Viking atmospheric carbon dioxide measurements: ▲, 600–1,200 °C steps measured; ◆, 900–1,200 °C steps measured; ■, trapped atmospheric component calculated. Allan Hills 77005 (AH); Chassigny (C); Elephant Moraine 79001-lithologies A, B and C (EA, EB, EC); Gobernador Valadares (GV); Lafayette (L); Nakhla (N); three Shergotty samples (S, Sb, Sc) and Zagami (Z).

most easily discernible in EETA79001 lithology C. Note that about 60% of the excess carbon observed for the Shergotty sample (S) combusts at 700 °C with a $\delta^{13}\text{C}$ of -23% , suggesting the presence of a third component.

The glass from EETA79001 is distinct from other samples studied, in that carbon released by combustion above 600 °C (Fig. 1b) is enriched in ¹³C by $\sim 20\%$. Figure 2a shows the complete release profile for the glass; a similar profile for the host igneous material, lithology A, is shown in Fig. 2b for comparative purposes. The component which causes the glass (lithology C) to be enriched in ¹³C above 600 °C is the carbon with positive $\delta^{13}\text{C}$ values released between 1,000 and 1,200 °C which is absent from the host rock.

Other authors^{2,3} comparing samples of lithologies A and C of EETA79001 have noted the presence in the latter of noble gases and nitrogen with relative abundance and isotope compositions that match values measured for the martian atmosphere⁴ and have accordingly argued for the existence of a trapped atmospheric component in the glass phase. The release temperatures for the proposed atmospheric components are >1,100 °C (ref. 2) and >800 °C (ref. 3) respectively. Although no isotopically heavy carbon is seen in the lithology A sample, some carbon is liberated between 900 and 1,200 °C. We take this to be indigenous carbon of igneous origin. This does not signify that we believe no terrestrial contaminants survive above

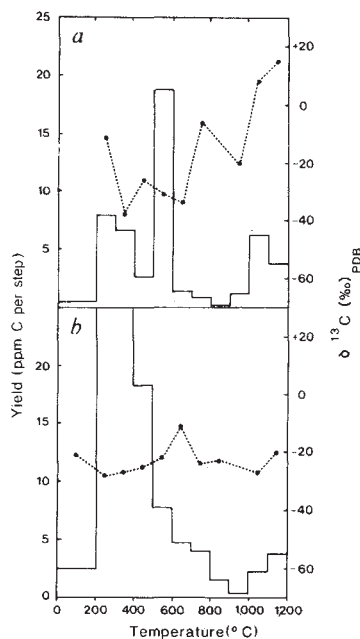


Fig. 2 Isotope composition and carbon release profile for stepped combustion of the Elephant Moraine shergottite 79001. *a*, Lithology C (glass); *b*, lithology A.

600 °C or, indeed, that indigenous components are not volatile at lower temperatures. Assuming this original carbon is completely retained during the process of shock melting, we calculate the difference between lithologies C and A (as done for nitrogen³) that is the trapped carbon component, to be 4.6 p.p.m. of carbon with an isotope composition of +36‰ ($\pm 10\%$) ignoring any small contribution from spallogenic sources. The abundance of noble gases (and nitrogen) measured in different glass inclusions, assuming an atmospheric origin, suggest that 5 p.p.m. of trapped martian carbon dioxide ought to be present². The agreement between predicted and measured abundance is extremely good. Likewise, the $^{13}\text{C}/^{12}\text{C}$ ratio corresponding to a $\delta^{13}\text{C}$ value of +36‰ is within the errors of the 'ground truth' measurements made by Viking spacecraft⁴.

The inset to Fig. 1*b* shows the experimental and calculated data for EETA79001 lithology C and the atmospheric isotope composition at the predicted abundance. These points fall on the trend established from >600 °C carbon measurements and seem to support the two-component model proposed above with: (1) the heavy carbon species being atmospheric carbon dioxide; (2) the indigenous igneous carbon, by implication, having a $\delta^{13}\text{C}$ value of about -30‰; (3) shergottites and nakhlites belonging to a common provenance and having a related origin; and (4) the universally shocked shergottites being more likely to exhibit an atmospheric component trapped in glass. In relation to point (4), excess amounts of ^{40}Ar , interpreted as being from the martian atmosphere have been found in other shergottite samples⁹ and we have previously pointed out the tendency for $\delta^{13}\text{C}$ values to rise at high temperatures for SNC meteorites¹⁰. A more convincing demonstration of the applicability of the two-component model requires separation and analyses of shock-produced glass from a variety of SNC specimens, although this may only be possible for ALHA77005.

Although not specifically related to the above discussion, other differences exist between the stepped combustion profiles of lithology A and C samples of EETA79001. A proportion of the total carbon (25%) is liberated below 500 °C for the glass; this is substantially less than for non-Antarctic meteorites and EETA79001 lithology A. The relatively high $\delta^{13}\text{C}$ value (-11‰) of the 200–300 °C extraction shows that this cannot be due entirely to biologically-produced organic contamination. Similar isotopically heavy carbon releases have been encountered with other Antarctic meteorite samples and it has been suggested that

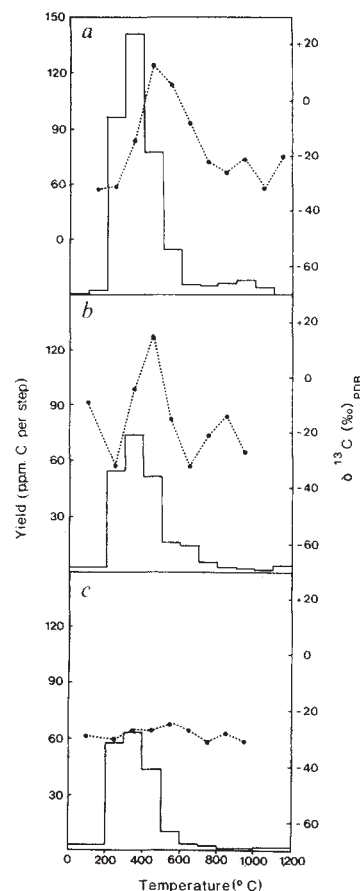


Fig. 3 Isotope composition and carbon release profiles for stepped heating: *a*, of Nakhla in oxygen; *b*, of Nakhla in *vacuo*; *c*, of the H_3PO_4 dissolution residue in oxygen.

a terrestrial weathering product may be responsible⁵. The component is presumably masked by more abundant isotopically light contamination in lithology A. Much more interesting is the large release of carbon with $\delta^{13}\text{C} = -31\%$ at 500–600 °C from lithology C but not lithology A. This component was also found for ALHA77005, another Antarctic shergottite. It may be connected with the excess of carbon in Shergotty because a larger sample of bulk Shergotty studied by Fallick *et al.*¹¹, also liberated substantial amounts of carbon at 600–700 °C with $\delta^{13}\text{C} = -28\%$. The source of this carbon is unknown; however, it may be related to the presence of shock-altered minerals, increased abundance in a large Shergotty specimen being consistent with a chance sampling of such material.

Nakhla has a bulk $\delta^{13}\text{C}$ of -12‰ quite distinct from all other SNC meteorites (Fig. 1*a*). This bulk isotope composition, first recognized by Fallick *et al.*¹¹, is dictated by the presence of a component which is volatile at <600 °C since Nakhla plots along with the other nakhlites in Fig. 1*b*. The individual steps of the stepped combustion (Fig. 3*a*) and a stepped pyrolysis (Fig. 3*b*) demonstrate that the component is, in fact, decomposed in the temperature range 300–500 °C, either with or without oxygen being present, to give carbon dioxide with a $\delta^{13}\text{C}$ rising to +15‰ in both cases. Treatment of powdered Nakhla with either hydrochloric or orthophosphoric acid removes the isotopically heavy carbon component as is shown by the stepped combustion of the residue of H_3PO_4 treatment (Fig. 3*c*). During the orthophosphoric acid reaction, carbon dioxide was collected in two steps and taken for isotope measurement. The results, given in Table 1, confirm that this gas contains carbon which is isotopically heavy, having $\delta^{13}\text{C}$ values up to +12‰. The thermal stability and chemical properties of the isotopically heavy carbon component in Nakhla are consistent with it being a carbonate present at ~0.02 wt % level. The particularly extended reaction time

Table 1 Acid dissolution of Nakhla

BM 1913,26 (43.17 mg)			BM 1911,370 (152.61 mg)		
Extraction time (h)	p.p.m. C	$\delta^{13}\text{C}$ (‰ PDB)	Extraction time (h)	p.p.m. C	$\delta^{13}\text{C}$ (‰ PDB)
18	21	+8	18	15	+6
24*	8	+12	48*	7	+46†
			24*	4	+49†
42	29	+9	90	26	+24

*Supplementary to original extraction time.

†Mass scans of these samples indicated the presence of organic species, which may have interfered with the isotope ratio measurements of the CO_2 . Hence, the determined $\delta^{13}\text{C}$ values may show an apparent enrichment in ^{13}C above the true value.

recognized during H_3PO_4 treatment of a sample of a second stone from the Nakhla meteorite shower (BM1911, 360) suggests that the species is not calcite but more probably dolomite or an iron-containing carbonate. Neither of the other nakhlites show any evidence of a ^{13}C -rich component decomposing at between 300 and 500 °C, although this may be because of the release of relatively large amounts of organic material from these contaminated samples. During the stepped combustions of one sample of Shergotty and the Chassigny specimen, $\delta^{13}\text{C}$ values did increase to -12‰ and -18‰ respectively¹⁰, but more work would be necessary to demonstrate the presence of carbonate.

A petrological investigation to confirm the presence of carbonate in Nakhla is required, but assuming that its existence is verified, the question which must be answered concerns its origin—terrestrial or martian? Terrestrial carbonates with $\delta^{13}\text{C}$ values higher than +10‰ are rare¹². Terrestrially produced carbonates are a common feature of Antarctic weathering; all appear to have normal $\delta^{13}\text{C}$ values but such features are less conspicuous in non-Antarctic meteorites⁵. For martian carbonate to survive in Nakhla, the shock events involved in its ejection from the planet surface would presumably need to be such that temperatures did not rise to greater than 500 °C.

We have speculated that carbon dioxide in the martian atmosphere is enriched in ^{13}C relative to carbon of presumed igneous origin as present in the SNC meteorites. It may be coincidence, but recognition of enrichments of the same order for the carbonate may be circumstantial evidence for an origin by weathering in the martian environment, particularly as dolomite would be an expected product of gas-solid reaction of martian pyroxenes¹³. We note, too, that Nakhla contains iddingsite, an aqueous alteration product of olivine, which is believed to be preterrestrial in origin¹⁴. Unfortunately, the pyrolysis gas chromatograph-mass spectrometer experiment¹⁵ carried out by Viking Landers, which might have been able to detect the decomposition products of carbonate and provide some isotope information was performed in such a way as to preclude such identification. It does not seem possible that the carbon dioxide trapped in EETA79001 is the relict of carbonate decomposed during shock heating, as such a route would be extremely unlikely to preserve the observed relative abundance to other atmospheric gases.

The existence of a trapped component of carbon dioxide in the correct abundance relative to noble gases and nitrogen and of an appropriate isotope composition to be of martian atmospheric origin strengthens the case that EETA79001 originated from our sister planet. The relationship between the martian atmospheric composition and data from EETA79001, other shergottites, and the nakhlites (inset in Fig. 1b) demonstrates that all these meteorites are related and presumably also derived from Mars. Likewise, demonstration of the existence of a carbonate mineral with a martian atmospheric composition would be direct evidence of the origin of Nakhla and its consorts from the planet.

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- Wood, C. A. & Ashwal, L. D. *Proc. Lunar planet. Sci.* **12B**, 1359-1375 (1981).
- Bogard, D. D. & Johnson, P. *Science* **221**, 651-654 (1983).
- Becker, R. H. & Pepin, R. O. *Earth planet. Sci. Lett.* **69**, 225-242 (1984).
- Owen, T. *et al. J. geophys. Res.* **82**, 4635-4639 (1977).
- Swart, P. K., Grady, M. M. & Pillinger, C. T. *Meteoritics* **18**, 137-154 (1983).
- Gibson, E. K. Jr & Bogard, D. D. *Meteoritics* **13**, 277-289 (1978).
- Gibson, E. K. Jr & Kotra, R. K. *Meteoritics* **17**, 220-221 (1979).
- DesMarais, D. J. *Proc. 9th Lunar planet. Sci. Conf.* 2451-2467 (1978).
- Bogard, D. D. *Abstr. 47th met. Soc. Meet., Albuquerque*, J-5 (1984).
- Carr, R. H., Wright, I. P. & Pillinger, C. T. *J. geophys. Res.* (in the press).
- Fallick, A. E. *et al. Abstr. 14th Lunar planet. Sci. Conf.* 183-184 (1983).
- Deines, P. *Geochim. cosmochim. Acta* **32**, 613-625 (1968).
- Gooding, J. L. *Icarus* **33**, 483-513 (1978).
- Bunch, T. E. & Reid, A. M. *Abstr. 37th met. Soc. Meet., Los Angeles* (1974).
- Kotra, R. K., Gibson, E. K. Jr & Urbancic, M. A. *Icarus* **51**, 593-605 (1982).

A high-temperature high-resolution NMR study of ^{23}Na , ^{27}Al and ^{29}Si in molten silicates

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Molecular and atomic motions over longer distances, and with much lower frequencies than interatomic vibrations, have a major role in the energetic and dynamic properties of highly structured liquids such as molten aluminosilicates. Rearrangement with increasing temperature of the polymerized anionic network (composed of linked SiO_4^{4-} and AlO_4^{5-} tetrahedra) controls viscosity and influences thermal expansion and configurational heat capacity^{1,2}. Diffusive motion of network modifying cations (such as Na^+) through the structure allows electrical conductivity and probably also affects entropy. To study such motion directly and to compare liquid and glass structure, we have developed a novel high-temperature high-resolution NMR apparatus. We report here the first data on ^{23}Na , ^{27}Al , and ^{29}Si in liquids in the system $\text{Na}_2\text{O}/\text{Al}_2\text{O}_3/\text{SiO}_2$ at temperatures to about 1,300 °C. Observed changes in NMR resonant frequencies (chemical shifts) give information on the effects of composition on local structure. Line shapes and widths, and relaxation time measurements, reveal details of the dynamics and of the transition from liquid to glass.

The apparatus developed for high-temperature liquid-state NMR will be described in detail elsewhere. A sample in a 1-cm diameter boron nitride capsule is rapidly shuttled 15 cm between a furnace and a room-temperature radio frequency NMR coil, spending 10 s in the former and ~0.5 s in the latter during each cycle, during which data from a single free-induction decay (FID) is recorded. Both the probe and the furnace are mounted in a 4.2 T superconducting magnet. Sample temperatures could not be measured during actual NMR experiments, but calibration runs indicate that reported values are accurate to within about 30 °C.

Samples were prepared from reagent grade oxides and carbonates. For all ^{29}Si NMR, 95% enriched $^{29}\text{SiO}_2$ was used. Analyses of unenriched samples after the NMR runs showed that by the end of runs of 6-12 h, Na_2O contents were reduced by 5-20% of the amount present. B_2O_3 contents became as high as 4 wt%.

Measurements were made with a home-built Fourier-transform NMR spectrometer, with 90° pulse lengths of 39 μs for ^{23}Na and ^{27}Al and 45 μs for ^{29}Si . Data from 40 FIDs were averaged per spectrum for ^{23}Na , and up to 400 for ^{27}Al and ^{29}Si .

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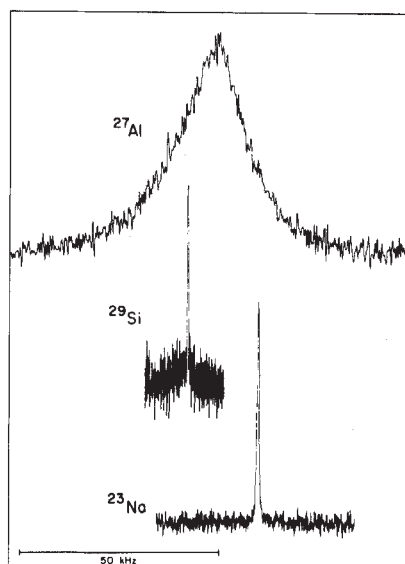


Fig. 1 Typical NMR spectra for molten silicates. Data are for $\text{NaAlSi}_2\text{O}_6$ at about 1,300 °C. Relative location of the spectra is arbitrary, but all have the same frequency scale.

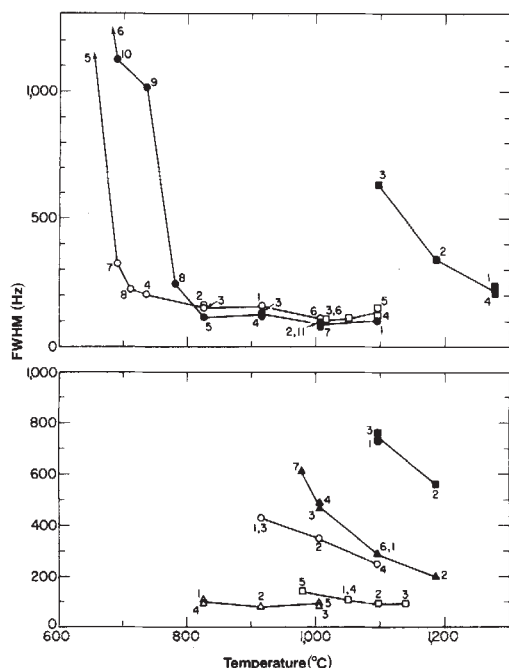


Fig. 2 Linewidths for ^{29}Si (top) and ^{23}Na (bottom) liquid spectra, as a function of temperature. Symbols indicate nominal composition: ●, $\text{Na}_2\text{Si}_4\text{O}_9$; ○, $\text{Na}_2\text{Si}_2\text{O}_5$; ■, $\text{NaAlSi}_2\text{O}_6$; □, Na_2SiO_3 ; ▲, 'R15' (see text); △, NaCl. Numbers show the order in which measurements were made and indicate reproducibility.

Relaxation time measurements were made by standard pulse techniques, using compensated π pulse sequences³. Chemical shifts are referenced to saturated aqueous NaCl (about 6.5 M), $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$, and TMS, all at room temperature.

Typical spectra are shown in Fig. 1, linewidths in Fig. 2 and chemical shifts in Fig. 3. The chemical shift measurements were made at the beginning of runs when compositions were presumed to be close to nominal.

As shown in Fig. 3, ^{29}Si chemical shifts (δ) change systematically with composition, as expected from data on glassy and crystalline solids⁴⁻⁷. Liquids that are more polymerized (higher mean Q^n number, where n is the number of other AlO_4 or SiO_4 units bound to a given SiO_4 tetrahedron) have more negative δ

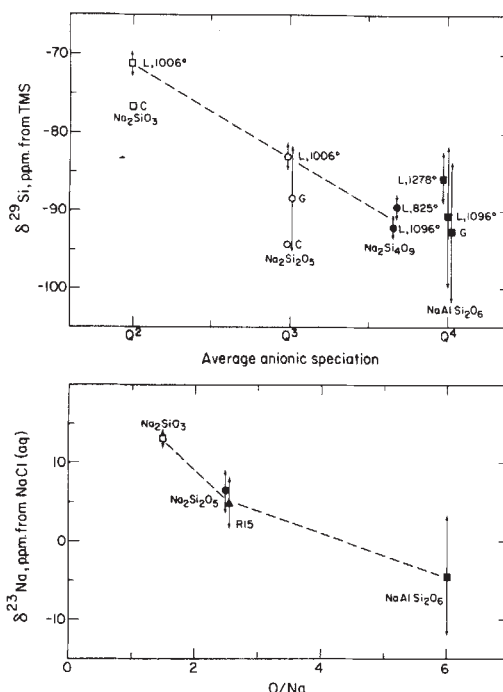


Fig. 3 Chemical shifts in silicates. Symbols as in Fig. 2, with L indicating liquid (at temperatures shown), C, crystalline solid, and G, glass (the latter two at room temperature). Arrows indicate linewidth, not errors. Q^n is the average speciation of the liquid, indicating n other (Si, Al) O_4 tetrahedral structural groups attached to a given group.

values, showing that ^{29}Si nuclei in tetrahedral sites with greater numbers of surrounding SiO_4 tetrahedra are more chemically shielded. Substitution of Al for some of the Si reduces this shielding, as shown for $\text{NaAlSi}_2\text{O}_6$ liquid and glass. Silicon chemical shifts in the liquids are systematically less negative (less shielding) than in the corresponding glasses and crystals.

Sodium chemical shifts in liquid silicates seem to have a simpler variation with composition. In all four silicate samples studied, $\delta(^{23}\text{Na})$ is well correlated with the ratio of the number of oxygen to sodium atoms, independent of polymerization state or the Al/Si ratio. Crystalline $\text{NaAlSi}_3\text{O}_8$ (−7.7 p.p.m.)⁸ falls very close to the trend for the liquids, when referenced to the same standard⁹. Our value of 6.8 p.p.m. for $\delta(^{23}\text{Na})$ in molten NaCl agrees well with data for the solid (6.7 p.p.m.)⁸.

Spectra for ^{27}Al in samples $\text{NaAlSi}_2\text{O}_6$ and R15 (Na_2O : 35, Al_2O_3 : 15, SiO_2 : 50 mol%) each contain only a single very broad feature (about 20 kHz wide), centred at $\sim 29 \pm 20$ p.p.m. The breadth of the peaks makes it difficult to draw conclusions about Al coordination in the liquids¹⁰.

Observed linewidths (FWHM) at the highest temperatures studied for both ^{23}Na and ^{29}Si are quite narrow (100–200 Hz, or 2–5 p.p.m.), with symmetrical lorentzian line shapes. Direct measurements of T_2 for ^{23}Na and ^{29}Si indicate that, for linewidths greater than about 200 Hz, the 'intrinsic' contribution from relaxation of spins dominates, and that, therefore, $\text{FWHM} \approx 1/(\pi T_2)$, where T_2 is the spin-spin relaxation time¹¹. Smaller linewidths are dominated by instrumental effects. A few T_1 (spin-lattice) relaxation times for ^{23}Na show that $T_1 \approx T_2$ in $\text{Na}_2\text{O}/\text{SiO}_2$ liquids, but that $T_1 > T_2$ in the more viscous aluminosilicate liquids. Narrow high-temperature linewidths for ^{29}Si indicate that silicate species move rapidly enough to average out the effects of chemical shielding anisotropy (at least 3.5 kHz)⁶, while ^{23}Na motion in the binary liquids is probably at least as fast as the NMR resonant frequency of the nucleus (or, the Larmor frequency, 47.3 MHz). For both nuclei, the FWHM increases (T_2 decreases) dramatically with decreasing temperature, as the motion that induces relaxation is 'frozen out'. For ^{29}Si , the lowest temperature at which data were

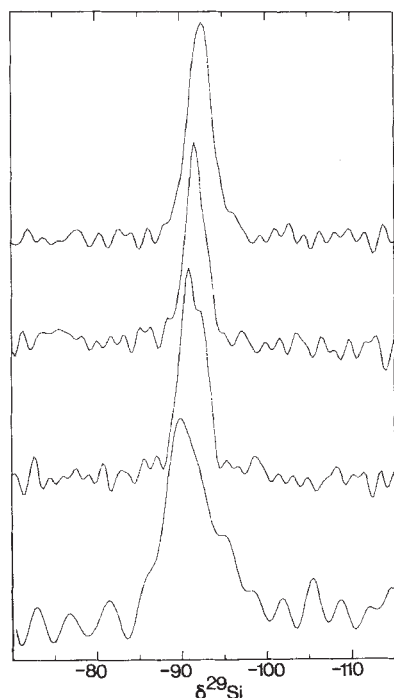


Fig. 4 ^{29}Si spectra for $\text{Na}_2\text{Si}_4\text{O}_9$ liquid at 1,096 °C (top), 1,006 °C, 915 °C, and 779 °C (bottom), normalized to the same peak height. $\delta(^{29}\text{Si})$ is in p.p.m. Note the development of a shoulder on the central peak as the temperature is lowered.

collected was that at which the spectrum became extremely broad and 'solid-like'. In $\text{Na}_2\text{Si}_4\text{O}_9$, this was probably the result of crystallization. The $\text{NaAlSi}_2\text{O}_6$ sample, however, was recovered as a glass, indicating that the 'glass transition' with respect to molecular motion faster than 3.5 kHz was observed. Not surprisingly, more-polymerized more-viscous liquids have wider NMR lines (shorter T_2 values) at a given temperature (Fig. 2).

The very broad bands observed for ^{27}Al indicate that even though these samples were liquid, quadrupole coupling was strong enough to cause the nuclei to relax very rapidly ($T_2 \approx 20 \mu\text{s}$).

For Na and Si in a series of silicate liquids at high temperatures, molecular motion has a significant component at or above the kHz frequency range. At lower temperatures, the loss of this motion can be observed. The nature of this motion remains a key problem. In a discussion of liquid heat capacities, Stebbins *et al.*² emphasized the possibilities of rotational and translational motion of anionic molecular groups, but pointed out that mechanisms involving the breaking of strong Si-O or Al-O bonds must become important in more polymerized liquids.

Raman and magic-angle spinning NMR spectra of glasses^{7,12} show that a range of silicate (Q^n) species, with a different range of chemical shifts for each value of n , make up the anionic structure of these materials. Observation of only one narrow ^{29}Si NMR line in the liquid suggests that these species chemically-exchange rapidly with respect to the typical separation in resonant frequency between the species (10–15 p.p.m. or 300–500 Hz). Only a time-averaged spectrum is observed. In aqueous silicate solutions, similar effects are seen, until temperature is lowered enough and exchange rates become slow enough to allow peak splitting to occur¹³. The development of shoulders on ^{29}Si peaks in some silicate melt spectra may suggest a similar slowing of reaction rates (Fig. 4). Structural rearrangement that includes the exchange of Q^n species involves breaking and reforming of Si-O bonds and apparently occurs even in a rather depolymerized liquid such as Na_2SiO_3 . The relative contribution of this type of motion to the overall energetics of the liquid remains unknown.

The preliminary results presented here thus show that NMR spectroscopy on silicate liquids can help to identify what types

of molecular motion occur, can begin to quantify the rates of such motions, and demonstrates the effects of composition. These and further data should prove useful in the understanding of both the thermodynamic properties of melts and the closely related processes of flow and diffusion.

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1. Richet, P. *Geochim. cosmochim. Acta* **48**, 471–483 (1984).
2. Stebbins, J. F., Carmichael, I. S. E. & Moret, L. K. *Contr. Miner. Petrol.* **86**, 131–148 (1984).
3. Levitt, M. H. & Freeman, R. J. *Magn. Res.* **43**, 65–80 (1981).
4. Lippmaa, E., Magi, M., Samoson, A., Englehardt, G. & Grimmer, A.-R. *J. Am. chem. Soc.* **102**, 4889–4893 (1980).
5. Lippmaa, E., Magi, M., Samoson, A., Tarmak, M. & Englehardt, G. *J. Am. chem. Soc.* **103**, 4992–4996 (1981).
6. Smith, K. A., Kirkpatrick, R. J., Oldfield, E. & Henderson, D. M. *Am. Miner.* **68**, 1206–1215 (1983).
7. Murdoch, J. B., Stebbins, J. F. & Carmichael, I. S. E. *Am. Miner.* (in the press).
8. Kundla, E., Samoson, A. & Lippmaa, E. *Chem. phys. Lett.* **83**, 229–232.
9. Templeman, G. J. & Van Greet, A. L. *J. Am. chem. Soc.* **94**, 5578–5582 (1972).
10. de Jong, B. H. W. S., Schramm, C. M. & Parziale, V. E. *Geochim. cosmochim. Acta* **47**, 1223–1236 (1983).
11. Farrar, T. C. & Becker, E. D. *Pulse and Fourier Transform NMR, Introduction to Theory and Methods* (Academic, New York, 1971).
12. McMillan, P. *Am. Miner.* **69**, 622–644 (1984).
13. Englehardt, G. & Hoebbel, D. *JCS Chem. Commun.* 514–516 (1984).

Alteration of basalt glasses: implications for modelling the long-term stability of nuclear waste glasses

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The long-term stability of nuclear waste form borosilicate glasses must be predicted from extrapolations of short-term laboratory data and then verified, which is only possible by comparing the glasses with their natural analogues of geological age. Here, we suggest that basalt glasses are useful analogues for predicting the long-term behaviour of nuclear waste form borosilicate glasses. Previous workers^{1–11} have considered the possibility of using natural glasses as analogues, but our study is a direct comparison of the experimentally studied corrosion products of basalt glasses, their natural long-term corrosion products and those of borosilicate glasses. Observations on the alteration processes for natural basalt glasses are combined with the experimental data on the alteration of basalt glasses and borosilicate glasses and are used to generate radioactivity release curves for nuclear waste form borosilicate glasses using the QTERM computer code^{12–15}. Our data on natural glasses place significant constraints on the anticipated fraction of activity released; major differences between our two release curves do not occur until after 1,000 years.

Hydrothermal leaching experiments were completed on three glasses: two basalt glasses (KL 9 and 11) from Kilauea, Hawaii ($\text{SiO}_2 = 50 \text{ wt}\%$, 24 kyr old) and a simulated borosilicate nuclear waste glass, C-31-3EC-SPF-Na ($\text{SiO}_2 = 35 \text{ wt}\%$). We report here the results of experiments at 200 °C in saturated synthetic NaCl solution for 30 days. The basalt glasses were remelted before leaching and their chemical composition determined by electron probe analysis (EPA) (Table 1). The compositions of the surface layers and solutions were determined by optical emission spectroscopy (ICP) and the surfaces examined by scanning electron microscopy. The compositions of a basalt glass and its palagonite surface layer (deep-sea dredge sample, USNM 113715, from

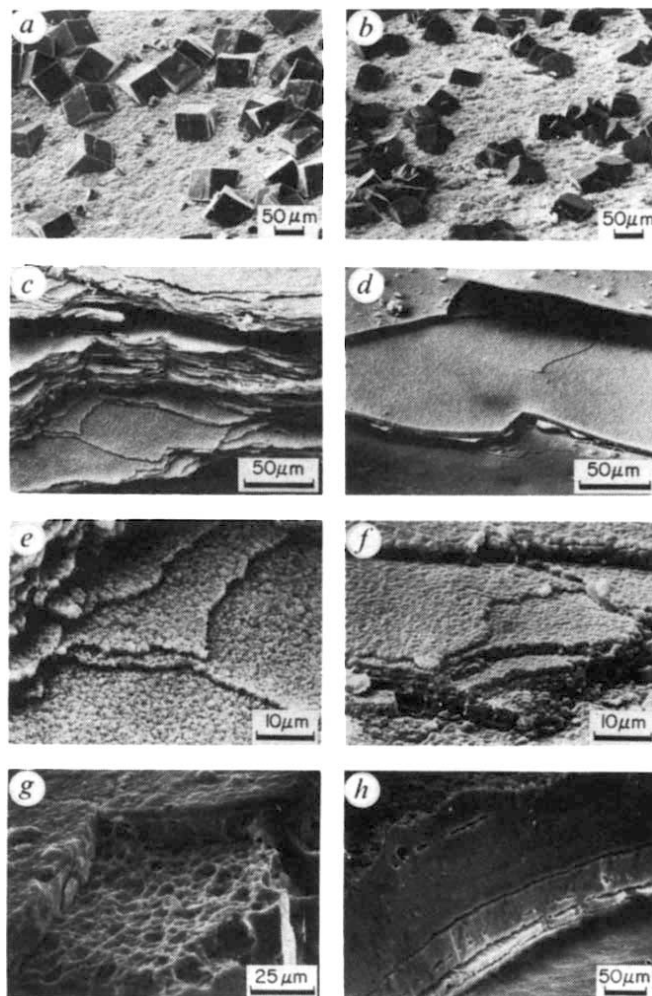


Fig. 1 Scanning electron micrographs of surface of basalt glass, KL 11, (a, c, e) and borosilicate nuclear waste glass (b, d, f) leached in saturated synthetic NaCl solution at 200 °C for 30 days. Dredge sample of basalt glass, USNM 113715, (g, h), altered in sea water at ~3 °C.

the Mid-Atlantic Ridge near the Kane Fracture zone, 2889–2475-m depth) are listed in Table 1 for comparison with our experimental results. This basalt glass is almost identical in composition to the normal Mid-Ocean Ridge Basalt (MORB) and the surface layer is similar to the 'average' palagonite cited by Staudigel and Hart (Table 2 of ref. 16). The dredge sample was altered by natural processes at low temperatures (~3 °C) in contact with sea water.

The most striking evidence for the similarity in the corrosion behaviour of basalt glass (KL 11) and waste form borosilicate glass (C-31-3-EC) is shown in the series of scanning electron micrographs (Fig. 1). In both cases, isometric analcime crystals were formed as surface precipitates (Fig. 1a, b). At higher magnifications, the repetitive sheet-like structures are apparent for both (Fig. 1c, d) and, at the highest magnification (Fig. 1e, f), each sheet is covered by a mat of crystal rosettes (probably clay). More importantly, a similar multiple surface layer structure (Fig. 1e, f) is common in naturally altered (~3 °C, sea water) basalt glasses (Fig. 1g, h). The same type of surface morphology on naturally altered basalt glasses has been well illustrated in previous studies^{8,17,18}.

The chemical similarities in the corrosion process are summarized in Tables 1 and 2. Table 1 compares the concentrations of major constituents of surface layers with the corresponding unaltered glass for basalt glasses, a borosilicate glass and a basaltic glass dredge sample. Note that in Table 1 we report only major constituents for comparative purposes (additional

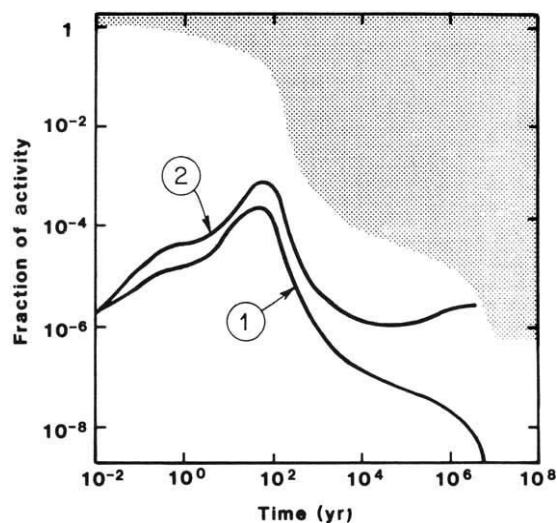


Fig. 2 Fraction of activity inventory released from the glass at any time (radioactive decay and thermal history of the waste form, with maximum temperature of 90 °C, included in the calculation of the curves). Case 1 is for parabolic time dependence; case 2 is for linear time dependence.

constituents are indicated in the footnotes). For this reason the totals of the analyses are not given, but we assume that the most important component that would bring the analyses to almost 100% is structural water. For example minerals such as vermiculite (an identified phase in surface layers) have water contents as high as 20 wt%. Although it is not possible to give totals for the analyses (because of the undetermined water content), their veracity is confirmed by mass-balance calculations (Table 2). All surface layers show depletion in SiO₂ and CaO, alumina (Al₂O₃) concentrations are approximately constant and there are increases in the concentrations of TiO₂, FeO, MnO and Na₂O in the surface layers. For the experimentally corroded glasses, the basalt glass and borosilicate glass behave similarly in terms of the amount of altered glass (as determined by weight loss and solution chemistry) and calculated depth of reaction (Table 2). For all glasses, surface layer formation is related to glass composition. The higher the ratios of Mg, Al, Fe and Ti to Si in the glass, the higher is the fraction of the surface layer relative to the total reacted glass (Table 2). The mass balance of the solution analyses and the surface analyses can be evaluated by comparing the total amount of altered glass (weight loss measurement with surface layer removed) with the sum of the altered material in the surface layer (weight measurement of surface layer) and the principal elements found in solution (SiO₂, Al₂O₃ and CaO). The data (Table 1) on the naturally altered basalt glass are used to illustrate the degree to which the experimental results of rather short-term experiments (30 days) match those for alteration processes and products of natural basalt glasses of much greater age. Our results for a naturally altered basalt glass are similar to those reported elsewhere^{19–23}. There are, however, variations in the observations made among natural basalt glasses that are due to the variety of conditions, time periods and processes of alteration (see particularly Table 1 of ref. 23).

Finally, if the alteration processes and products of natural basalt glasses are satisfactory analogues of what one expects for the long-term alteration of borosilicate glasses, can this information be used to generate (or verify) models used to predict the behaviour of borosilicate glasses? Figure 2 illustrates this application by using the QTERM code (described in refs 12–15) to generate the fraction of radioactivity released for two test cases. The boundary of the shaded area in the upper portion of the figure represents the decay of the radionuclides in the canister and marks the maximum fraction of released activity if there were no borosilicate glass waste form and all activity

Table 1 Major constituents in unaltered glasses and their surface layers

Constituents (wt %)	Basalt KL 9		Basalt KL 11		Borosilicate glass C-31-3EC		Basalt USNM 113715 (dredge sample)	
	Glass (EPA)	Surface (ICP)	Glass (EPA)	Surface (ICP)	Glass‡ (EPA)	Surface§ (ICP)	Glass (EPA)	Surface (EPA)
SiO ₂	50.2	25.2	50.0	24.2	34.7	18.0	50.4	41.2
Al ₂ O ₃	13.2	10.8	10.0	9.8	11.8	12.1	15.3	17.9
TiO ₂	2.6	3.8	1.8	2.8	3.4	5.5	1.9	1.9
Fe ₂ O ₃	11.1*	18.6	12.6	16.6	0.5	0.9	10.8*	14.0*
MnO	0.16	0.3	0.15	0.21	0.22	0.26	0.19	<0.05†
MgO	7.8	11.1	12.0	12.6	1.4	2.0	7.7	3.1
CaO	10.9	0.5	7.8	0.25	3.6	0.88	10.8	1.23
BaO	—	—	—	—	13.6	3.0	—	—
K ₂ O	0.4	—)†	0.65	—)†	—	—	0.14	2.4
Na ₂ O	2.2	5.4	3.6	4.1	4.8	9.2	3.0	1.6

Conditions: 200 °C, 30 days in NaCl solution, surface area/solution volume = 0.014 cm⁻¹.

* Reported as FeO.

† Below detection limit.

‡ Glass contains 22% waste oxides and 4.1% B₂O₃.

§ Data for 60 days. Layer contains 9.7% waste oxides, 5.7% ZrO₂ and 1.4% ZnO.

|| Sample in contact with sea water at ~3 °C for geological periods of time (Mid-Atlantic Ridge).

Table 2 Summary of glass leach data

	Basalt KL 9	Basalt KL 11	Borosilicate C31-3-EC
Weight loss measurement (g m ⁻²)			
Surface layer removed	80	134	72
Surface layer	47†	92†	30†
Elements analysed in solution (g m ⁻²)			
SiO ₂	24	32	16
Al ₂ O ₃	1	1	5
CaO	8	13	15‡
Others	—	—	6§
Glass constituents retained in surface layer	Na, Al, Si as analcime, Mg*, Fe*, Ti*	Na, Al, Si as analcime, Mg*, Fe*, Ti*	Na, Al, Si as analcime, Mg*, Fe*, Ti* and some fission products
Density (g cm ⁻³)	2.837	2.862	3.265
Calculated depth of reaction (µm)	28	47	22

* In unidentified compounds.

† Includes uptake of bound H₂O and Na.

‡ Σ (CaO + BaO).

§ Σ (of oxides of Li, Zn, B, Mo, Sr).

were released immediately on contact with water. The experimental data for both cases are taken from data published by the joint research project of Japan, Switzerland and Sweden²⁴ where a radioactive glass with an SiO₂ content of 45 wt% was used. Experiments were performed in water at 90 °C following the MCC-1P test procedure²⁵. The complete composition and experimental conditions are summarized elsewhere²⁴. Data are available for up to 182 days. The essential questions are what model of corrosion is applicable, and how widely will results vary as a function of the model selected? In case 1, the time dependence is assumed to be parabolic, $t^{1/2}$ (refs 26, 27), where the most important consideration in the corrosion of the waste glass is the formation of a 'protective' layer that then becomes a diffusion barrier to further release. Beyond the 182-day database, we have continued the parabolic time dependence which has been used to describe the hydration of rhyolitic glasses²⁸. In case 2, the time dependence is linear, t^1 , based on the analysis of Si and B data²⁴, assuming an initial first-order dissolution reaction for the glass with silica approaching saturation. Between 28 and 182 days, glass corrosion proceeded at a low but constant rate as indicated by boron release. Beyond the 182-day database, we have continued the linear time dependence, as required in describing the formation of palagonite rinds on basalt glasses²⁹. The latter case is the most conservative (maximum) estimate of the released activity. The curves for cases 1 and 2 are only illustrative (as the temperature dependence

of palagonitization of natural basalt glass and the alteration of waste glasses is still under investigation), but they do provide us with important examples of time-limited experimental data combined with information derived from the alteration of natural glasses over long periods of time.

Three important points may be made. (1) Depending on the model used to extrapolate the long-term release of radionuclides, there are wide variations in the fraction of released activity (for example, at 10 kyr, an order of magnitude). (2) There is little difference between the two cases in the fraction of activity released during the first 100 yr, but afterwards there is an increasing difference. At 1 Myr, for case 2, there is little difference between the fraction of activity released and the total fraction of remaining activity due to radioactive decay (the intersection of the release curve with the shaded area), whereas at 1 Myr, for case 1, the waste form is still able to reduce the fraction of activity released by two orders of magnitude. (3) Data on naturally altered basalt glasses (that is, time dependence of corrosion, role of layer formation in release of elements and identification of stable phases in the precipitates) can be used in presently available release codes (QTERM). The use of the appropriate natural analogue is of immense value in designing and verifying release codes over long periods of time.

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1. Ewing, R. C. *Scientific Basis for Nuclear Waste Management* Vol. 1 (ed. McCarthy, G.) 57-68 (Plenum, New York, 1979).
2. Ewing, R. C. & Haaker, R. F. *Naturally Occurring Glasses: Analogues for Radioactive Waste Forms* (Battelle Rep. PNL-2776/UC-70, 1979).
3. Adams, P. B. *Int. Symp. Ceramics in Nuclear Waste Management* (eds Chikalla, T. D. & Mendel, J. E.) 233-237 (American Ceramic Society, 1979).
4. Malow, G. & Ewing, R. C. *Scientific Basis for Nuclear Waste Management* Vol. 3 (ed. Moore, J. G.) 315-322 (Plenum, New York, 1981).
5. Dickin, A. P. *Nature* **294**, 342-345 (1981).
6. Mellinger, P. J., Vemparti, C. S., Allnatt, A. R. & Jacobs, P. W. M. *Physics and Chemistry of Glasses* **22**, 49-54 (1981).
7. Bates, J. K., Jardine, L. J. & Steindler, M. J. *Science* **218**, 51-53 (1982).
8. Allen, C. C. *Scientific Basis for Nuclear Waste Management-V* (ed. Lutze, W.) 37-44 (Elsevier, New York 1982).
9. Barkatt, A. et al. *Geochim. cosmochim. Acta* **48**, 361-372 (1984).
10. Plodinec, M. J., Jantzen, C. M. & Wicks, G. G. *2nd Int. Symp. Ceramics in Nuclear Waste Management* (eds Wicks, G. G. & Ross, W.) 491-495 (American Ceramic Society, 1984).
11. Malow, G., Lutze, W. & Ewing, R. C. *J. non-crystalline Solids* **67**, 305-321 (1984).
12. Altenhein, F. K., Lutze, W. & Ewing, R. C. *Scientific Basis for Nuclear Waste Management-V* (ed. Lutze, W.) 45-56 (Elsevier, New York, 1982).
13. Altenhein, F. K., Lutze, W. & Ewing, R. C. *Scientific Basis for Nuclear Waste Management-VI* (ed. Brookins, D.) 269-280 (Elsevier, New York, 1983).
14. Altenhein, F. K., Lutze, W. & Ewing, R. C. *2nd Int. Symp. Ceramics in Nuclear Waste Management* (eds Wicks, G. G. & Ross, W.) 636-644 (American Ceramic Society, 1984).
15. Freude, E., Grambow, B., Lutze, W., Rabe, H. & Ewing, R. C. *Scientific Basis for Nuclear Waste Management-VIII* (eds Jantzen, C. M., Stone, J. A. & Ewing, R. C.) (Elsevier, New York, in the press).
16. Staudigel, H. & Hart, S. R. *Geochim. cosmochim. Acta* **47**, 337-350 (1983).
17. Allen, C. C., Gooding, J. L., Jercinovic, M. J. & Keil, K. *Icarus* **45**, 347-369 (1981).
18. Jercinovic, M. J. thesis, Univ. New Mexico (1984).
19. Ailin-Pyzik, I. B. & Sommer, S. E. *J. geophys. Res.* **86**, 9503-9510 (1981).
20. Melson, W. G. *Trans. Am. geophys. Un.* **54**, 1011-1014 (1973).
21. Melson, W. G. & Thompson, G. *Bull. geol. Soc. Am.* **84**, 703-716 (1973).
22. Schmincke, H.-U. *Init. Rep. DSDP LXV*, 477-483 (1983).
23. Honnorez, J. *The Sea* (ed. Emiliani, C.) 525-587 (Wiley, New York, 1981).
24. Hermansson, H.-P., Björner, L.-K., Christensen, H. & Yokoyama, H. *Studvik Rep. NW-84/653* (1984).
25. MCC-1P, Static Leach Test Method *Nuclear Waste Materials Handbk* (US Department of Energy, DOE/TIC-11400, 1981).
26. Wicks, G. G. & Wallace, R. M. (Savannah River Laboratory, DOE Rep. DP-82-18, 1982).
27. Wicks, G. G. & Wallace, R. M. *Scientific Basis for Nuclear Waste Management-VI* (ed. Brookins, D.) 23-28 (Elsevier, New York, 1983).
28. Friedman, I., Smith, R. L. & Long, W. D. *Bull. geol. Soc. Am.* **77**, 323-328 (1966).
29. Morgenstein, M. & Riley, T. J. *Asian Perspectives* **17**, 145-159 (1974).

Flow law for ice in polar ice sheets

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Theories of glacier flow are based commonly on the assumption that ice is not a newtonian fluid, but has a non-linear stress-dependent viscosity¹⁻⁵. Here we re-examine the spreading of Antarctic ice shelves and suggest that the data cannot define a unique flow law. Tilt measurements in four boreholes in both the Arctic and Antarctic seem to show that a linear flow law $\dot{\epsilon} = A_1 \tau$ (where $A_1 \sim 10^{-15} \text{ s}^{-1} \text{ Pa}^{-1}$ at -28°C), where $\dot{\epsilon}$ is the effective strain rate and τ the effective shear stress, may be just as appropriate for describing the flow of polar ice sheets.

Conventional flow laws for ice are written as $\dot{\epsilon} = A_n \tau^n$. A_n is often assumed to be modelled adequately by an Arrhenius temperature dependence, $A_n = A_0 \exp(-Q/RT)$, where Q is the activation energy, R the gas constant and T the absolute temperature. To allow for a possible pressure dependence, T is referred to the *in situ* melting point. A_0 is assumed to include the effects of parameters that describe variations in flow, such as grain size, ice fabric and impurity content. According to laboratory experiments, the exponent n can range from 1 at low stresses (below $\sim 10^4 \text{ Pa}$) to 4 or even higher at stresses $> 10^5 \text{ Pa}$ (ref. 6), in agreement with field data for borehole closure⁷, where $n = 3$ was found for $\tau = 2 \times 10^5 - 10^6 \text{ Pa}$, although these latter data may include a large component of transient creep.

The stresses, that are important for ice-sheet flow, however, lie in the range $10^4 - 10^5 \text{ Pa}$. Data from spreading of ice shelves have been interpreted⁸ as showing that, in this stress range, n is ~ 3 , which is now the generally accepted value, although Whillans⁹, using an empirical formulation of the flow law derived from tilting of boreholes, found the stress exponent varied between 1 and 2. Low values of n at low stresses have

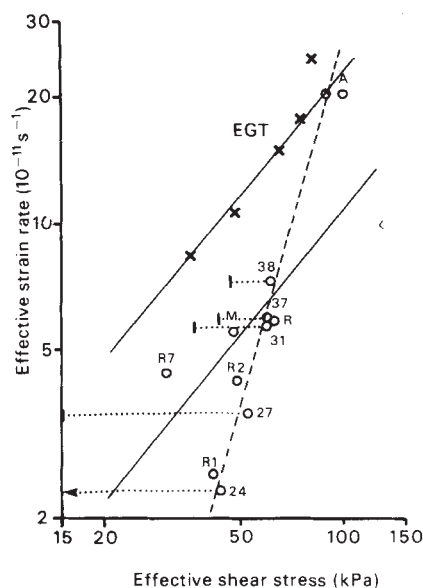


Fig. 1 Plot of effective strain rate against effective stress for ice-shelf sites⁸ (A = Amery; M = Maudheim; R = Ross; R1, R2, R7, 24, 27, 31, 37, 38 = Brunt), with an $n = 3$ line (dashed). Error bars show the possible influence of the restraining force from MacDonald Ice Rumples. Solid line is an $n = 1$ line that falls within the error bars. The uppermost line and crosses are for Erebus Glacier Tongue¹¹.

been explained as the effects of transient creep. Paterson^{3,10}, reviewing laboratory and field data, has shown that there is a large spread in experimental results, such that under similar conditions strain rates may vary by more than an order of magnitude; but by selecting measurements thought to be most reliable, he could recommend³ values of $n = 3$, $Q = 60 \text{ kJ mol}^{-1}$ and $A_3 = 5.2 \times 10^{-25} \text{ s}^{-1} \text{ Pa}^{-3}$ at -10°C . Further analysis of borehole data has revealed that Holocene ice in both Greenland and Antarctica was ≤ 20 times softer than expected from ice-shelf spreading data¹⁰. We have attempted to reduce this discrepancy by re-examining ice shelf and borehole data from several sites, without assuming *a priori* that $n = 3$. We have not attempted to determine a flow law in the rheological sense, but only to find those values for the above parameters that give the best agreement with the field results.

Ice-shelf data (strain rates and ice thickness) were used by Thomas⁸ for sites selected to conform as closely as possible to the ideal of an unbounded ice shelf. Being unable to measure the stress field directly, he calculated the effective shear stress averaged over depth by assuming that there was no restraint by lateral shear at side walls and ice rises and that the only longitudinal restraint was by sea water pressure acting along the direction of the maximum principal strain rate. Figure 1 shows the effective strain rates plotted against effective shear stresses. Thomas⁸ stated that a flow law with $n = 3$ fitted the data. Not only were all the sites constrained laterally to a greater or lesser extent, thus giving lateral shear restraint, but also the Brunt Ice Shelf sites, which are the main constraint on the $n = 3$ line, were influenced by MacDonald Ice Rumples. Using his derived flow law, Thomas⁸ calculated a significant restraining force exerted by MacDonald Ice Rumples on Brunt Ice Shelf. Although for more distant sites the direction of the force is almost perpendicular to the direction of the maximum principal strain rate and may therefore be considered to have been accounted for by the lateral strain rate, there could still be a lateral shear restraint. Because of this uncertainty in describing the stress field, we show in Fig. 1, for the relevant Brunt Ice Shelf site, error bars on the effective shear stress whose magnitudes are derived from Thomas's plot of restraining force against distance from MacDonald Ice Rumples⁸. The linear flow law line $\dot{\epsilon} = A_1 \tau$, which passes through the data from Maudheim and Ross Ice Shelf, lies well within these error bars.

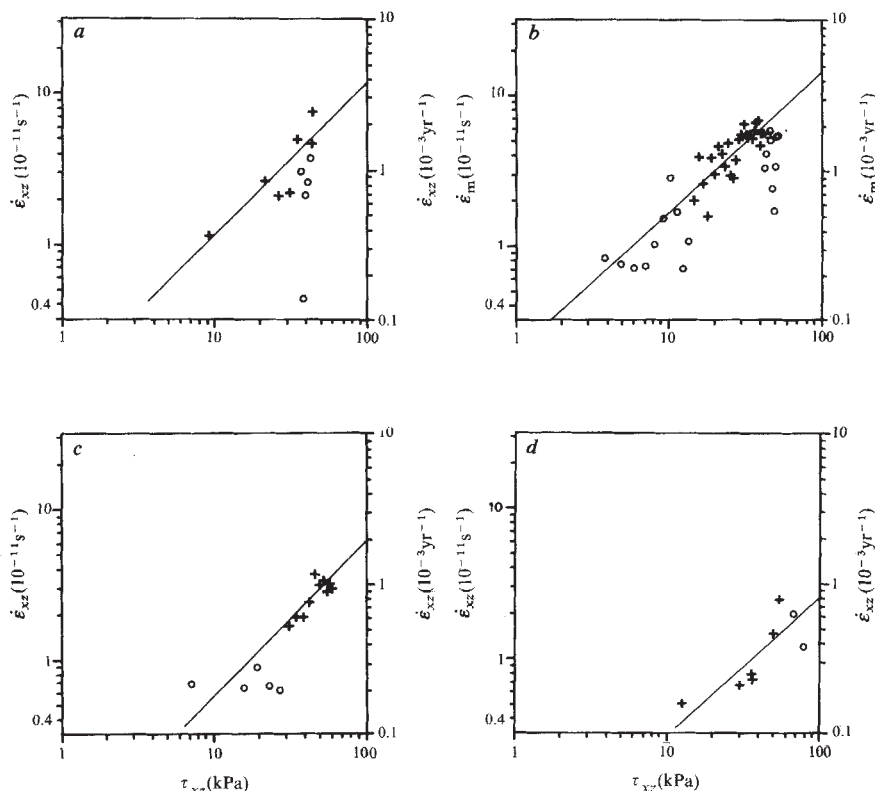


Fig. 2 Logarithmic plots of $\dot{\epsilon}_{xz}$ against τ_{xz} from the tilting rate of boreholes. *a*, Camp Century; *b*, Byrd Station; *c*, Devon Island; *d*, Law Dome. The Byrd Station and Law Dome data are corrected as described in the text. Data used in the regression are marked as crosses, the rest as circles and the line of best fit is shown.

Erebus Glacier Tongue (EGT) is perhaps a closer approximation to an unbounded ice shelf. Holdsworth¹¹ interpreted its spreading by assuming that $n = 3$, and he was forced to postulate large variations in the value of A_3 . We have plotted these data on Fig. 1. With the exception of the point of highest stress, which is only 2 km from the hinge line and where the velocity field (and therefore strain rate) is uncertain, the points fall closely on a line with $n = 1$. At the mean estimated temperature of -13°C , the value of A_1 is $2.6 \times 10^{-15} \text{ s}^{-1} \text{ Pa}^{-1}$. In summary, it seems that, because of the uncertainty in the stress fields, the ice-shelf data cannot be used to determine a unique flow law, although the ice shelf that is closest to being unbounded is that which most strongly suggests a linear law.

Tilt measurements have been made over several years to near bedrock at Camp Century^{10,12} in Greenland and Devon Island¹³ in Arctic Canada, and for about the top 70% of the ice thickness at Byrd Station^{10,12} and Law Dome¹⁴ in Antarctica. Paterson¹⁰ has plotted a restricted data set for Camp Century and Byrd Station on a logarithmic plot of $\dot{\epsilon}_{xz}$ against τ_{xz} where the axes x, y, z define a rectangular coordinate system, x is the direction of flow and z is vertically downwards. He obtained a reasonable fit to curves computed using $n = 3$, but only when using a value of A_3 15–20 times that previously recommended. But for these few scattered data an equally good or better fit is obtained if curves are computed using $n = 1$. In Fig. 2*a–d* we have plotted for the four sites all the data, referred to a temperature of -28°C , using an activation energy of 60 kJ mol^{-1} .

For Camp Century and Devon Island, we have simply plotted the temperature-compensated $\dot{\epsilon}_{xz}$ against τ_{xz} , but for Byrd Station, flow in the upper part of the hole can be perpendicular to the surface gradient and in Fig. 2*b* we used, following Paterson¹⁰, a resolved strain rate $\dot{\epsilon}_m = (\dot{\epsilon}_{xz}^2 + \dot{\epsilon}_{yz}^2)^{1/2}$. The reason for the spiralling flow at Byrd is unknown, but it seems reasonable to follow earlier investigators in assuming that the flow, in whatever direction, is driven ultimately by shear stresses related to the surface slope. For Law Dome, laboratory measurements have been made¹⁴ of the relative softness of ice from different depths. Because the relevant experiment used strain rates determined at a given stress and temperature, it does not rely on the assumption of any flow law. The variations in softness were related to ice fabric and were reported as enhancement factors

(*f*) between 1 and 4. In Fig. 2*d*, we have plotted $\dot{\epsilon}_{xz}/f$ compensated to -28°C , to remove, where possible, variations in flow resulting from factors other than stress dependence; the two highest stress points were obtained from an extrapolation of strain rates to bedrock, assuming zero basal velocity¹⁴.

As we have allowed for temperature change, we might expect the data in Fig. 2 to fall on a straight line with gradient n , the flow law exponent. Quasi-linearity (that is, gradient = 1 on this plot), however, would be observed for a power law in regions where longitudinal stresses dominate. Therefore, in our regression analysis to find the best value of n , we have discarded all data above the greatest depth (for each site) at which the resolved longitudinal strain rate (taken as the measured surface value, Table 1) is greater than $\dot{\epsilon}_{xz}$ (or $\dot{\epsilon}_m$). Following Paterson¹⁰, we have included neither the data for Wisconsin ice, as its flow could be affected by other factors, nor the two extrapolated points at Law Dome. Regressions on the remaining data (shown by crosses in Fig. 2) gave n values of: 1.04 for Camp Century, 0.93 for Byrd Station, 1.03 for Devon Island and 0.8 for Law

Table 1 Measured surface longitudinal strain rates

	Camp Century ¹⁰	Byrd Station ¹⁰	Devon Island ¹⁶	Law Dome ¹⁷
Strain rates (10^{-4} yr^{-1})				
$\dot{\epsilon}_{xx}$	2.6	4.4	6.7	2.3
$\dot{\epsilon}_{yy}$	0	1.4	0.2	5.7

Table 2 Values and ratios of A_1 for ice shelves and boreholes (at -28°C)

	Erebus Glacier Tongue	Camp Century	Byrd Station	Devon Island	Law Dome
A_1 ($\text{s}^{-1} \text{ Pa}^{-1}$)	4.8×10^{-16}	1.5×10^{-15}	1.4×10^{-15}	5.4×10^{-16}	2.1×10^{-16}
Ratio:	1	3	3	1	0.4

Law Dome has been corrected for laboratory measured enhancement factors; the value and ratio should be doubled to be compared with other sites.

Dome. Equivalent analyses using an activation energy of 40 kJ mol^{-1} give similar results, with n between 0.95 and 1.2 for the four sites.

Our results must be qualified because the difficulty in making accurate inclination measurements means that, generally, tilt data are of fairly poor quality, especially in the upper part of the borehole. Regressions through even the deeper data, however, would still give $n \ll 3$. This, together with the fact that all four boreholes suggest the same result, indicates that random errors in the poor quality data have not influenced the result unduly. In the upper layers of an ice sheet there may also be a component of transient creep resulting from the steadily increasing shear stress as the ice moves downwards, but this will also be a feature of the flow of the ice sheet as a whole, and is therefore a factor which a realistic analysis should not exclude.

Our second method of analysis allows explicitly for the effect of longitudinal stresses. For each datapoint we have calculated the value of the flow-law constant A_n normalized to a given temperature (-28°C) by

$$A_n = \left(\frac{\dot{\epsilon}_{xz}}{\rho g \alpha z} \right)^n \exp \left[\frac{Q_n}{R} \left(\frac{1}{T} - \frac{1}{T_0} \right) \right] / E^{n-1} \quad (1)$$

where n is the stress exponent (either 1 or 3), $\dot{\epsilon}_{xz}$ the vertical-shear strain rate, ρ the density, g the acceleration due to gravity, z the depth, α the surface slope, Q_n the activation energy, $T_0 = -28^\circ\text{C}$ and E^2 the second invariant of the strain-rate tensor. Local variability in the values of A results from unmodelled factors such as grain size, fabric or impurities. The general trend for all sites, however, is of a constant value of A_1 for a linear law, with the average A_1 in the top half of each hole very close to that found in the bottom half. Alternatively, for a power law ($n=3$), A_3 shows a consistent decrease with depth over several orders of magnitude. If a power law had been most appropriate at all depths, the trend should have been for a constant value of A_3 and an increasing value of A_1 with depth.

If we assume a linear law applies, then values of A_1 averaged over depth are given by the gradient of plots of $\dot{\epsilon}_{xz}$ against τ_{xz} on linear axes. Using the same data as before gives the values in Table 2; a value for Erebus Glacier Tongue is also given, corrected to a temperature of -28°C using an activation energy of 60 kJ mol^{-1} . The values agree to within a factor of four, compared with the factor of 15–20 needed by Paterson¹⁰ to reconcile borehole and ice-shelf data using a power law with $n=3$.

Another way of comparing flow laws is to consider horizontal velocity profiles with depth. These are obtained by integrating the measured borehole shear rates downwards from the surface to the lowest point at which they were measured. If the surface velocity is known, then an upper bound can be placed on the resulting deformation in the lowest unmeasured layers. At Byrd Station, extrapolating the average value of A_1 to the bed and using a linear law suggests a sliding velocity of 1.4 m yr^{-1} , which is reasonable because of the water at the bottom of the borehole¹⁵. Using $n=3$ and the average value of A_3 calculated for the last 250 m of measured tilting gives a negative velocity at the bed. There must be another reduction in A_3 (by an order of magnitude) for the basal velocity to be zero. At Law Dome, to avoid negative basal velocities calculated using $n=3$, Russell-Head and Budd¹⁴ suggested that shear stresses reached a maximum at two-thirds depth and then decreased towards the bed. This postulate is unnecessary with $n=1$, where values of A_1 can be extrapolated to the bed to give zero velocity there.

For all four boreholes, a linear law gives overall a more coherent explanation of the data and one which agrees better with ice-shelf data. If $n=3$ were the correct rheological exponent, then such large variations in A_3 would be required (with depth and site) that the flow law would cease to have any predictive value. We conclude that on evidence available at present it is not necessary to use a non-linear flow law to describe the flow and deformation of polar ice sheets.

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1. Nye, J. F. *Proc. R. Soc. A* **219**, 477–489 (1953).
2. Glen, J. W. *Proc. R. Soc. A* **228**, 519–538 (1955).
3. Paterson, W. S. B. *The Physics of Glaciers* 2nd edn (Pergamon, Oxford, 1981).
4. Hooke, R. le B. *Rev. Geophys. space Phys.* **19**, 664–672 (1981).
5. Weertman, J. A. *Rev. Earth planet. Sci.* **11**, 215–240 (1983).
6. Lile, R. C. *ANARE Sci. Rep.* No. 132 (1984).
7. Paterson, W. S. B. *Rev. Geophys. space Phys.* **15**, 47–55 (1977).
8. Thomas, R. H. *J. Glaciol.* **12**, 55–70 (1973).
9. Whillans, I. M. *J. geophys. Res.* **86**, 4272–4282 (1981).
10. Paterson, W. S. B. *Cold Regions Science and Technology* **8**, 165–179 (1983).
11. Holdsworth, G. *Ann. Glaciol.* **3**, 131–137 (1982).
12. Robin, G. de Q. in *The Climatic Record in Polar Ice Sheets* (ed. Robin, G. de Q.) 98–118 (Cambridge University Press, 1983).
13. Paterson, W. S. B. & Waddington, E. D. *Rev. Geophys. space Phys.* **22**, 123–130 (1984).
14. Russell-Head, D. S. & Budd, W. F. *J. Glaciol.* **24**, 117–130 (1979).
15. Ueda, H. T. & Garfield, D. E. *Int. Ass. Sci. Hydrol. Publ.* **86**, 53–62 (1970).
16. Paterson, W. S. B. *J. Glaciol.* **17**, 3–12 (1976).
17. MacLaren, W. A. *ANARE Sci. Rep. Ser. A(IV)* No. 103 (1968).

Sulphate sequestered in the sulphate-binding protein of *Salmonella typhimurium* is bound solely by hydrogen bonds

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An important question in understanding substrate binding by proteins is how charged groups are stabilized in the absence of their solvation shell. We have addressed this question here by solving the structure of the sulphate-binding protein of *Salmonella typhimurium* with bound substrate at 2.0 Å resolution. The results are remarkable in that the charged oxygen atoms of the sulphate molecule, which is buried and completely inaccessible to the solvent, are not stabilized by the formation of salt-bridges but by hydrogen bonds donated by specific residues of the protein. These hydrogen bonds are in turn coupled via peptide units to several resonating hydrogen bonding systems. These findings may be of general significance for the role of electrostatic interactions in protein structure and function.

In addition to the sulphate-binding protein, we have recently refined the molecular structures of three other binding proteins specific for L-arabinose¹, D-galactose and Leu, Ile or Val (N. K. Vyas, M. N. Vyas, J. S. Sack and F. A. Q., unpublished data; see also refs 2, 3). These periplasmic proteins, with dissociation constants of $\sim 10^{-6} \text{ M}$, belong to the family of proteins which act as initial receptors for active transport systems and chemotaxis in bacteria⁴. All four structures are ellipsoidal and composed of two similar globular domains connected by a flexible hinge.

The tertiary structure of the sulphate-binding protein at pH 4.3, which consists of 2,570 non-hydrogen atoms from 308 residues, 1 sulphate and 143 ordered water molecules, was refined by the method of Hendrickson and Konner⁵ to an R-factor of 0.14 for the 16,959 reflections from 8 to 2.0 Å. The refined structure differs from ideal bond lengths and angle distances by overall r.m.s. deviations of 0.015 Å and 0.038 Å, respectively. A more detailed description of the structure determination and protein conformation will be presented elsewhere.

Figure 1 shows the location of the binding site and Fig. 2 and Table 1 illustrate the precise details of sulphate binding. The sulphate is buried in the cleft ~ 7 Å below the protein surface and becomes completely inaccessible to the solvent; none of the $73\text{-}\text{\AA}^2$ accessible surface area⁶ of the free sulphate is exposed. There are no positively charged residues, cations or water molecules within van der Waals' distance of the sulphate, thus precluding the formation of salt-bridges to neutralize the charged sulphate. Instead, all of the sulphate oxygens are the recipients of hydrogen bonds from seven residues distributed

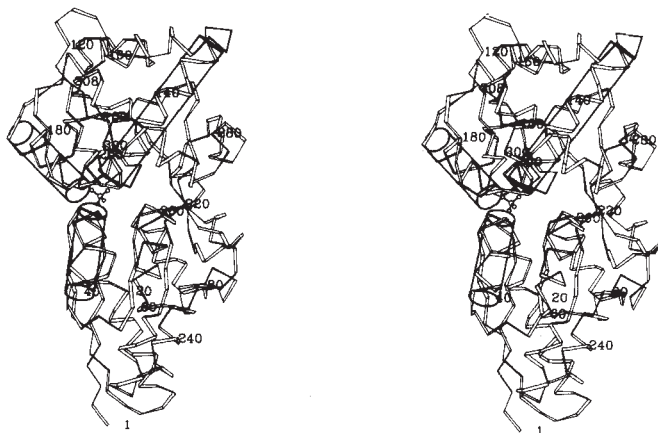


Fig. 1 Stereo view of the $C\alpha$ backbone trace of the sulphate-binding protein, with the sulphate bound in the cleft between the two domains. The sulphate is shown in a ball-and-stick model and the three helices (44–54, 131–146, 172–181) close to the sulphate are further enclosed in cylinders. The residues hydrogen-bonded to the sulphate originate from both domains: the N-terminal domain supplies Asp 11 and Ser 45 and the C-terminal domain provides Ser 130–Gly 131–Gly 132–Ala 133, Ala 173 and Trp 192. Because of four likely changes to the published sequence of 310 residues¹⁵ as a result of the present refinement, the sequence numbering scheme used in this study is based on the refined structure. However, as these changes (none in the binding site) consisted of one insertion, one deletion and two amino-acid substitutions, the total number of amino acids is unaltered. The last two residues are highly disordered. (Drawing produced by ARPLOT¹⁶.)

in both domains. These H-bonding residues are also buried. The sequence Ser 130–Gly 131–Gly 132–Ala 133, which forms part of a loop and the first turn of a helix, has a key role in binding, as the first three residues provide a complementary three-point docking site for O-1, O-2 and O-3 of the sulphate, respectively, and the Ala 133 peptide NH donates a hydrogen bond to the Ser 130 hydroxyl (see Fig. 2). The peptide bonds with CO groups from residues 44, 131 and 172 initiate three α -helices (Figs 1, 2b).

What neutralizes or stabilizes the sequestered dianion charges? Stabilization could be provided by the inducible hydrogen-bond dipoles, by the three helix macrodipoles (44–54, 131–146, 172–181) close to the sulphate (with opposite polarity) (Figs 1, 2b) or by a combination of the two. We propose that fixed hydrogen bond dipoles radially oriented around the sulphate, induced through polarization by the strong electric field of the dianion and further coupled (via peptide units) to several resonating or relay H-bond systems (see Fig. 2b), could effectively neutralize the charges. One of these systems, which is composed of two H-bonds (between O-2 of the sulphate and Gly 131 peptide NH and between Ser 130 peptide CO and Arg 134 NE), could dissipate the buried charges. Arg 134, which is 5.7 Å to the sulphate, is further involved in an inter-domain bidentate salt-link with Asp 68. Another relay system is formed by hydrogen bonding between O-4 of the sulphate and NH of Asp 11 and between CO of Tyr 10 and His 42 ND1, which is in contact with the solvent. A similar mechanism for dissipating charges could be achieved by three separate peptide bonds, each providing an NH H-bond to a sulphate oxygen and initiating via the CO group a 'zig zag' continuous sequence of alternating peptide units and H-bonds within the α -helix. The left-handed helical H-bond array has the form $O_i \cdots NH_{i+4}$, where $i = m + 3(n - 1)$; m is the residue bearing the first O and $n = 1, 2, 3 \dots$. An α -helix has three such arrays; ones of interest in SBP have m values of 44, 131 and 172.

As the Ser 130 hydroxyl is polarized by the dianion and is the recipient of a hydrogen bond from 133 NH located at the N-terminus of helix 131–146, the hydroxyl proton could be

Table 1 Hydrogen bonds between sulphate and binding protein

Protein atoms, donor (X)	Sulphate atoms, acceptor (Y)	Distance (Å) X...Y	Angle (°) X-H...Y
Ser 130 OG	O-1	2.76	167
Ala 173 N	O-1	2.70	151
Ser 45 N	O-2	2.84	169
Gly 131 N	O-2	2.83	170
Gly 132 N	O-3	2.77	149
Trp 192 NE1	O-3	2.82	157
Asp 11 N	O-4	2.67	152
Overall mean:		2.76 (0.07)	159 (9)

Hydrogen bonds were determined on the basis of angle and distance criteria and hydrogen atoms were located on the basis of standard geometry (see ref. 1).

shared with the O-1 of the sulphate, yielding transient HSO_4^- species (the pK_1 and pK_2 of the sulphuric acid are ~ 3 and 1.9, respectively⁷). Proton sharing could be further facilitated by the coupling of 133 NH with another resonating H-bond array ($m = 132$).

Helix macrodipoles have been implicated in anion binding at the positive N-terminus of macrodipoles⁸. It has been calculated that binding of a single negative charge 5 Å from the helix macrodipole N-terminus involves a substantial attractive energy contribution of 12 kcal mol⁻¹, which is minimally diminished by desolvation energy⁸ (this value may be overestimated significantly)⁹. The three helices in the sulphate-binding protein close to the dianion could provide charge stabilization. The effect of the macrodipoles may be diminished as the sulphate is not centred on any helix axis. These helices and several others with their N-termini converging towards the binding site cleft could also promote long-range attraction of anions preparatory to actual tight binding⁸. This would seem to be generally applicable to binding proteins except that several of the substrates are uncharged sugars and amino acid zwitterions.

The mechanism we have described above confines the function of an α -helix primarily to a specific H-bonded peptide array and takes into account resonances and polarizability of the array in dissipating buried charges. The mechanism would be more effective when the array is exposed to the solvent. This is what we observed in the amphipathic¹⁰ helices with m values of 44 and 172; the third helix is partially buried. Further, we have made similar observations from other proteins (for example, flavodoxin) known to bind anions at N-termini of amphipathic helices (unpublished findings). In this connection, it has been shown that carbonyl oxygens within the hydrophilic side of helices are often H-bonded to solvents¹¹.

The proposed function of a specific H-bond helical array could, in much the same way as it might enhance the sharing of a serine hydroxyl proton with the sulphate, increase the reactivity of enzyme active-site residues or of coenzymes hydrogen-bonded to the first turn of helices.

The structure of flavodoxin resembles one of the domains of the periplasmic binding proteins (G.L.G., M.A.S. and F.A.Q., unpublished data). Although the substrate site of flavodoxin is located in a crevice at the surface¹² (coordinates obtained from Protein Data Bank¹³), we observed some similarities in the binding of the phosphate moiety of the buried flavin mononucleotide with the sulphate binding described above. (1) Of the 10 H-bond donors to the phosphate oxygens, 5 are from peptide NH groups of loops and the N-terminus of the one helix (10–26) near the phosphate dianion; (2) a Ser OH simultaneously donates a H-bond to the phosphate and accepts a H-bond from a peptide NH; (3) the one line of peptide H-bonds ($m = 10$) is exposed to the solvent. As the phosphate is not deeply buried within the flavodoxin, several relay systems as found in SBP may not be required. Nonetheless, the carbonyl oxygens of peptides bearing the NH groups H-bonded to the phosphate

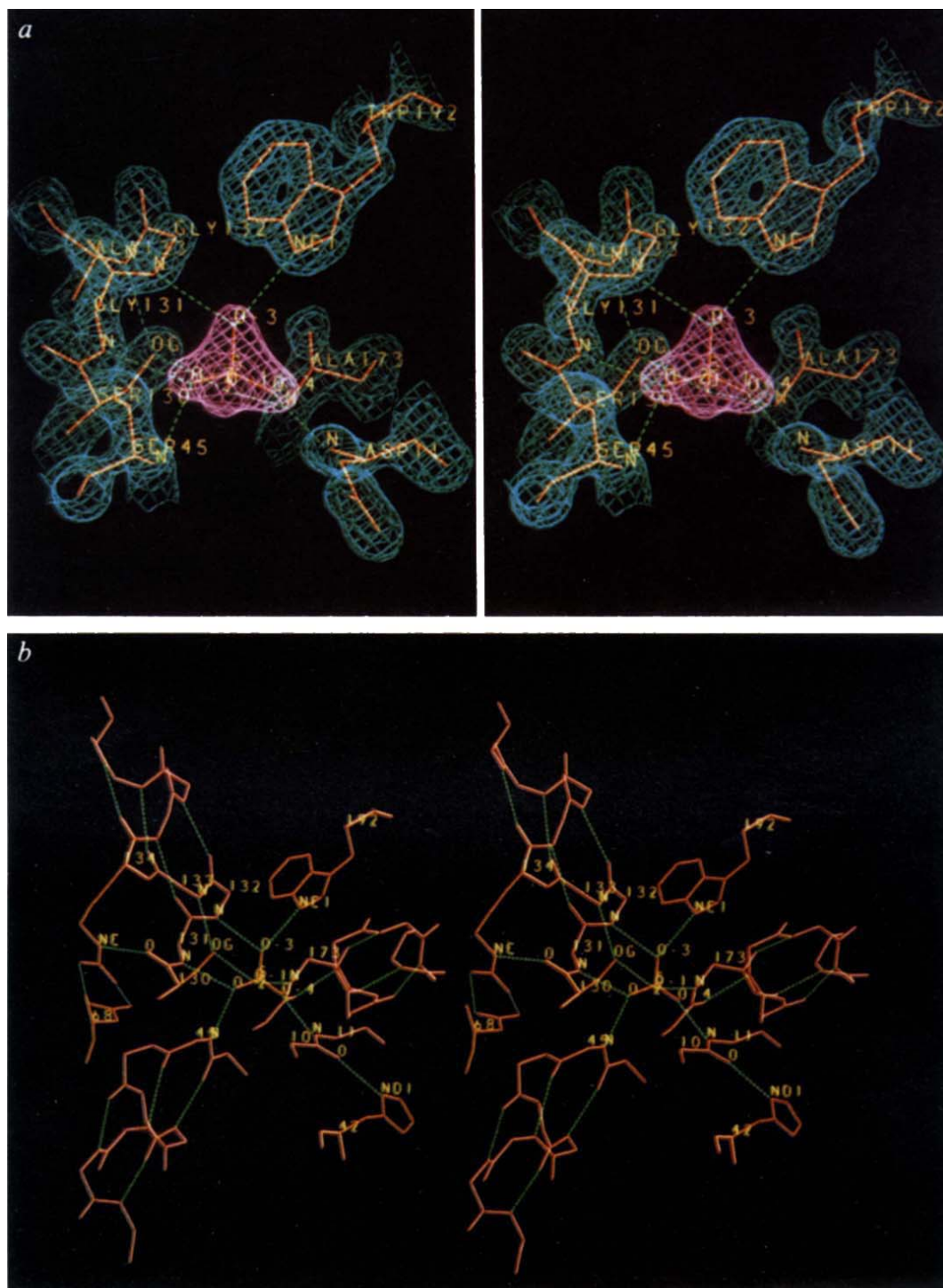


Fig. 2 *a*, Stereo view of the electron density of the tetrahedral sulphate and essential residues of the sulphate-binding protein superimposed on the refined model. The density map was calculated with coefficients $(2|F_o| - |F_c|)$ and final $2\text{ \AA}$ refined α_c . The density surface (sulphate in purple and amino acids in blue) was contoured at 1.5 times the estimated standard deviation of the electron density and at grid-spacing of $0.4\text{ \AA}$. The green dotted lines represent hydrogen bonds. The H-bonds formed between the sulphate and the sulphate-binding protein are listed in Table 1. The α -carbons are identified with the amino-acid name and number. The plane formed by Ser 130 OG, Gly 131 N and Gly 132 N is parallel to and maximally superimposed on the plane of O-1, O-2 and O-3 of the sulphate. *b*, The same as *a* except that the electron density surface and residue names have been removed for clarity. Furthermore, other hydrogen-bonding systems (described in the text) and at least the first turn of three helices close to the sulphate have been added. The molecular graphics program FRODO¹⁷ extensively modified for the E & S PS300 system¹⁸ was used in the refinement and in generating these figures.

are in contact with the solvent.

Despite completely different substrate specificities, there are similarities in the binding sites of the L-arabinose-binding protein¹ and the sulphate-binding protein. The L-arabinose is also bound in the cleft via several H-bonds from six buried residues in both domains and is inaccessible to the bulk solvent. It is

notable, especially in view of the importance of peptide bonds in anion binding, that while only side chains are involved in hydrogen-bonding the sugar in the arabinose receptor, five of the seven H-bonds to the sulphate are from the peptide backbone. (We have also observed that the charge of the solvent-inaccessible Arg 151, one of three essential charged residues in

the arabinose-binding protein, is stabilized entirely by protein and sugar dipoles¹). As H-bonds are highly directional, they confer geometrical specificity on the site and ensure correctness of fit for the substrate. It may also be important that these bonds are stable enough to provide significant binding but are of sufficiently low strength to allow rapid ligand dissociation. The estimated turnover rates of binding proteins *in vitro* are comparable to the $V_{\max}$ values for *in vivo* transport¹⁴. Transport could be impeded if the sulphate were bound with salt-links.

The novel finding of general significance presented here is

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1. Quijcho, F. A. & Vyas, N. K. *Nature* **310**, 381-386 (1984).
2. Vyas, N. K., Vyas, M. N. & Quijcho, F. A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1792-1796 (1983).
3. Saper, M. A. & Quijcho, F. A. *J. biol. Chem.* **258**, 11057-11062 (1983).
4. Landick, R. & Oxender, D. L. in *Bacterial Transport* (ed. Martonosi, A. N.) 81-88 (Plenum, New York, 1982).
5. Hendrickson, W. A. & Konner, J. H. in *Computing in Crystallography* (eds Diamond, R., Ramaseshan, S. & Venkatesan, K.) 13.01-13.23 (Indian Academy of Sciences, International Union of Crystallography, Bangalore, 1980).
6. Lee, B. K. & Richards, F. M. *J. molec. Biol.* **55**, 517-526 (1971).
7. Pauling, L. *The Nature of the Chemical Bond*, 325 (Cornell University Press, Ithaca, 1966).
8. Hol, W. G. J., van Duijnen, P. T. & Berendsen, H. J. C. *Nature* **273**, 443-446 (1978).

that charges from as strong an acid as sulphate can be stabilized in an enclosed, solvent-inaccessible site within a protein by bonds other than salt-links. This particular result and other observations reported here have far-reaching importance in understanding not only other ion-binding and transport systems, but protein stability, enzyme catalysis and other biochemical processes that require charged intermediates.

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9. Rees, D. C. *J. molec. Biol.* **141**, 323-326 (1980).
10. Perutz, M. F., Kendrew, J. C. & Watson, H. C. *J. molec. Biol.* **13**, 669-678 (1965).
11. Blundell, T., Barlow, D., Borkakoti, N. & Thornton, J. *Nature* **306**, 281-283 (1983).
12. Smith, W. W., Burnett, R. M., Darling, G. D. & Ludwig, M. L. *J. molec. Biol.* **117**, 195-225 (1977).
13. Bernstein, F. C. *et al. J. molec. Biol.* **112**, 535-542 (1977).
14. Miller, D. M. III, Olson, J. S., Pflugrath, J. W. & Quijcho, F. A. *J. biol. Chem.* **258**, 13665-13672 (1983).
15. Ishihara, H. & Hogg, R. W. *J. biol. Chem.* **255**, 4614-4618 (1980).
16. Lesk, A. M. & Hardman, K. D. *Science* **216**, 539-540 (1982).
17. Jones, T. A. in *Computational Crystallography* (ed. Sayre, D.) 3303-3317 (Clarendon, Oxford, 1982).
18. Pflugrath, J. W., Saper, M. A. & Quijcho, F. A. in *Methods and Applications in Crystallographic Computing* (eds Hall, S. & Ashida, T.) 404-407 (Clarendon, Oxford, 1984).

Significance of enamel thickness in hominoid evolution

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Enamel thickness has assumed unique importance in the debate about the hominid status of *Ramapithecus*, despite the fact that there is little agreement about the meaning of 'thick enamel' or the significance of enamel thickness for hominoid taxonomy¹⁻¹⁹. My aim here is to evaluate the usefulness of enamel thickness and microstructure as characteristics for determining the relationships of the later Miocene hominoids, based both on a quantitative study of enamel thickness in extant hominoids and four species of later Miocene *Sivapithecus* (including '*Ramapithecus*') and on scanning electron microscope analysis of enamel microstructure²⁰. Four categories of enamel thickness are defined metrically here and have been related to enamel microstructure and developmental rates. Thin fast-formed (pattern 3) enamel represents the ancestral condition in hominoids; it increased developmentally to thick pattern 3 enamel in the great ape and human clade and that condition is primitively retained in *Homo* and in the fossil hominoid *Sivapithecus* (including '*Ramapithecus*'). Enamel thickness has been secondarily reduced in the African apes and also, although at a different rate and extent, in the orang-utan. Thick enamel, previously the most important characteristic in arguments about the earliest hominid, does not therefore identify a hominid.

The most useful measurement of the amount of enamel on a tooth would be the volume of the tissue. This could be expressed as a thickness by dividing the volume of enamel by the surface area of the enamel/dentine junction over which the enamel formed; this area is proportional to the number of ameloblasts which formed the enamel. Such a measurement can be approximated by dividing the area of the enamel cap exposed by a single saw cut through the tooth by the length of the enamel/dentine junction over which it formed (average enamel thickness, Fig. 1). Single linear measurements of enamel thickness were found to be poorly correlated with this value, and such studies¹⁵, especially those based on estimates from worn teeth⁴ or naturally fractured teeth^{21,22}, are of limited value²⁰.

Enamel thickness increases allometrically (with increasing body and tooth size) in anthropoid primates^{4,15}. Gorillas have absolutely thicker enamel than do chimpanzees (Fig. 1) in direct relation to body size²⁰, whereas orang-utans and humans have

Table 1 Relative enamel thickness of fossil and recent great apes

	Pan	Gorilla	Homo	Pongo	Sivapi- thecus
No. of specimens	14	17	13	17	9
Mean	10.10	10.04	22.35	15.93	19.71
Minimum	7.02	6.75	13.76	11.32	16.07
Maximum	13.31	13.39	32.26	20.45	22.68
Standard deviation	2.09	1.74	6.23	2.51	2.58
Mean low 95%	8.90	9.15	18.58	14.65	17.73
Mean high 95%	11.30	10.93	26.12	17.21	21.69
$\frac{c/e}{\sqrt[3]{b.wt}} \times 100$ (mean)	1.68	1.80	3.00	2.84	—

The sample studied included 24 unworn, or very lightly worn, molar teeth for each of *Pan troglodytes* (the chimpanzee), *Gorilla gorilla*, *Pongo pygmaeus* (the orang-utan) and *Homo sapiens*, and a smaller number of molars of *Hylobates*, *Sivapithecus sivalensis* (formerly called *Sivapithecus indicus*^{20,28}), *Sivapithecus punjabicus* (formerly '*Ramapithecus punjabicus*'^{4,20}), *Sivapithecus darwini* and *Sivapithecus alpani* (formerly referred to '*Ramapithecus wickeri*'^{20,22}). These were mainly cut longitudinally in the coronal plane through the mesial cusps to provide two surfaces for measuring enamel thickness. Examples of each species were sectioned in the sagittal plane to determine the correspondence of the tips of the dentine horns with the cusp tips. The teeth were cut using a diamond wafering machine (Isomet, Buehler Ltd) producing a cut 350 μ m wide. It has been shown that obliquity of section can exaggerate apparent enamel thickness²⁰, but the method used here ensures that measurements of enamel thickness were taken at or within a few micrometres of the ideal plane of section through the enamel cap, and even at worst no more than 175 μ m mesial or distal to the ideal (see Fig. 1 legend). Mean low 95% and mean high 95% refer to the 95% confidence limits for the mean values for these samples. The final row gives values, for the living species, of a relative enamel thickness index based on body weight (b.wt, data from Dr Ben Rudder), which provides the same ranking of enamel thickness.

relatively thicker enamel when scaled for body and tooth size (Fig. 1)²⁰. As body weight data are not available for fossil species, a dental estimate of body size must be used to scale average enamel thickness. Tooth crown measurements are of little use⁴ as they include two measurements of enamel thickness in their magnitude and are not independent variables. The best estimator of body size was the combined area of dentine and pulp in the same section on which enamel thickness was measured (see Fig. 1)²⁰. When combined with average enamel thickness (c/e), this produces a dimensionless index: relative enamel thickness = $(c/e \times 100)/\sqrt[3]{b}$, where c is the area of the

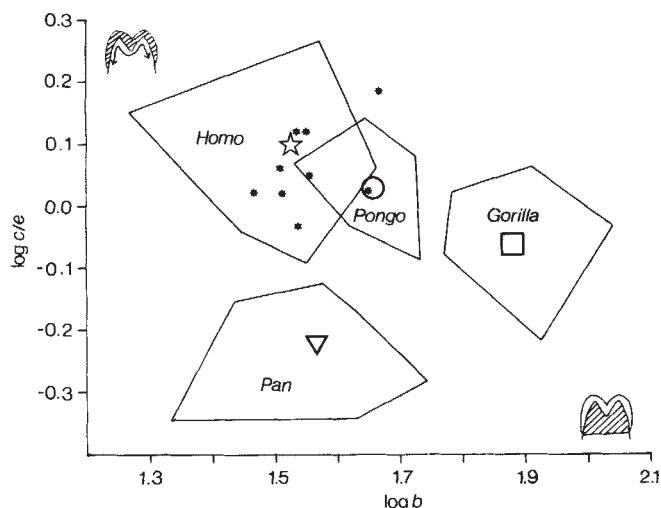


Fig. 1 Enamel thickness in relation to size. The vertical axis shows the average enamel thickness (c/e) and the horizontal axis the combined dentine/pulp area (b); both axes are logarithms to base 10. The shaded area of the tooth section shown on the y-axis is the enamel cap area (designated c) and the length of the interface between the shaded area and the unshaded area on the y-axis is the enamel/dentine junction length (e): average enamel thickness = c/e . The shaded area of the tooth section on the x-axis is the combined area of dentine and pulp (b), which was found to be the best dental approximator for body size that excluded any contribution from the enamel²⁰. The species ranges observed are shown as minimum area polygons, with the species means indicated. Fossil specimens attributed to four species of *Sivapithecus* are shown by site, ☆ representing specimens from the Siwaliks, Indo-Pakistan, and * representing specimens from Pasalar, Turkey.

Sample details are given in Table 1 and individual data points are given in ref. 20. Measurements for all upper and lower molars are combined, which could only increase the species ranges, as no significant differences in enamel thickness between different molars were found²⁰. Measurements were taken on photographs of the cut surface taken at constant magnification, with the cut face parallel with the film plane, and prints were also made at constant magnification²⁰. Area and perimeter measurements were made using a pen transducer attachment for the graphics pad of an Apple computer, and tracing errors were small²⁰. Linear measurements were taken to determine a good simple approximator of average enamel thickness. Correlations between average enamel thickness (c/e) and linear enamel thicknesses were $r=0.27-0.75$ for nine single linear dimensions in *Gorilla*²⁰; when two or three of these measurements were averaged before calculating the correlation, it increased to $r=0.74-0.76$ (ref. 20); and the correlation for the mean of all nine measurements on c/e was 0.85 (ref. 20). These results indicate the unreliability of single linear dimensions (contra Gantt¹⁵), and raise questions about the reliability of studies based on these^{15,19}, and even more so about studies based on estimates of linear dimensions of enamel thickness^{4,21}.

enamel cap, e is the length of the enamel/dentine junction over which the enamel formed and b is the combined area of dentine and pulp in the same section (Fig. 1). Table 1 gives values for this index together with values for a second index expressing enamel thickness relative to the cube root of body weight; the two dimensionless indices produce the same ranking of relative enamel thickness, although their actual values differ²⁰. The dentally based index has therefore been used as a measure of relative enamel thickness which is applicable to fossil species.

Four categories of relative enamel thickness are defined, based on the 95% confidence limits of the species means of relative enamel thickness (Table 1): thin enamel (species mean values of relative enamel thickness 8.90–11.30), intermediate/thin enamel (mean values 11.31–14.64), intermediate/thick enamel (mean values 14.65–17.49), and thick enamel (mean values 17.50–26.20). Thin enamel is found in *Pan*, *Gorilla* and *Hyllobates*, intermediate/thick enamel in *Pongo* and thick enamel in

Homo, and also in *Sivapithecus* (17.73–21.69).

These variations in enamel thickness can be explained in terms of the microstructure of hominoid enamel. Pattern 1 enamel²³ has previously been reported to occur in great ape enamel, while pattern 3 enamel is found in hominines²⁴. In contrast, Vrba and Grine²⁵ found pattern 3 enamel to be common in all hominoids, and Boyde and Martin²⁶ found that pattern 3 enamel predominates in the deep layers of hominoid enamel, sampled in developing surface preparations, although some pattern 1 enamel is always present superficially. The prevalence of pattern 3 enamel in hominoids has subsequently been recognized by Gantt¹⁹.

In the present work, sequential cuts made into the enamel provided information about enamel prism cross-sections, at a series of known depths, in the course of sectioning teeth for enamel thickness measurements^{20,26}. The cut surfaces (Fig. 2C) were examined by back-scattered scanning electron microscopy to give additional information about packing patterns in the deeper enamel, prism cross-striation repeat intervals, and the distribution of Hunter-Schreger bands (Fig. 2)^{20,26,27}.

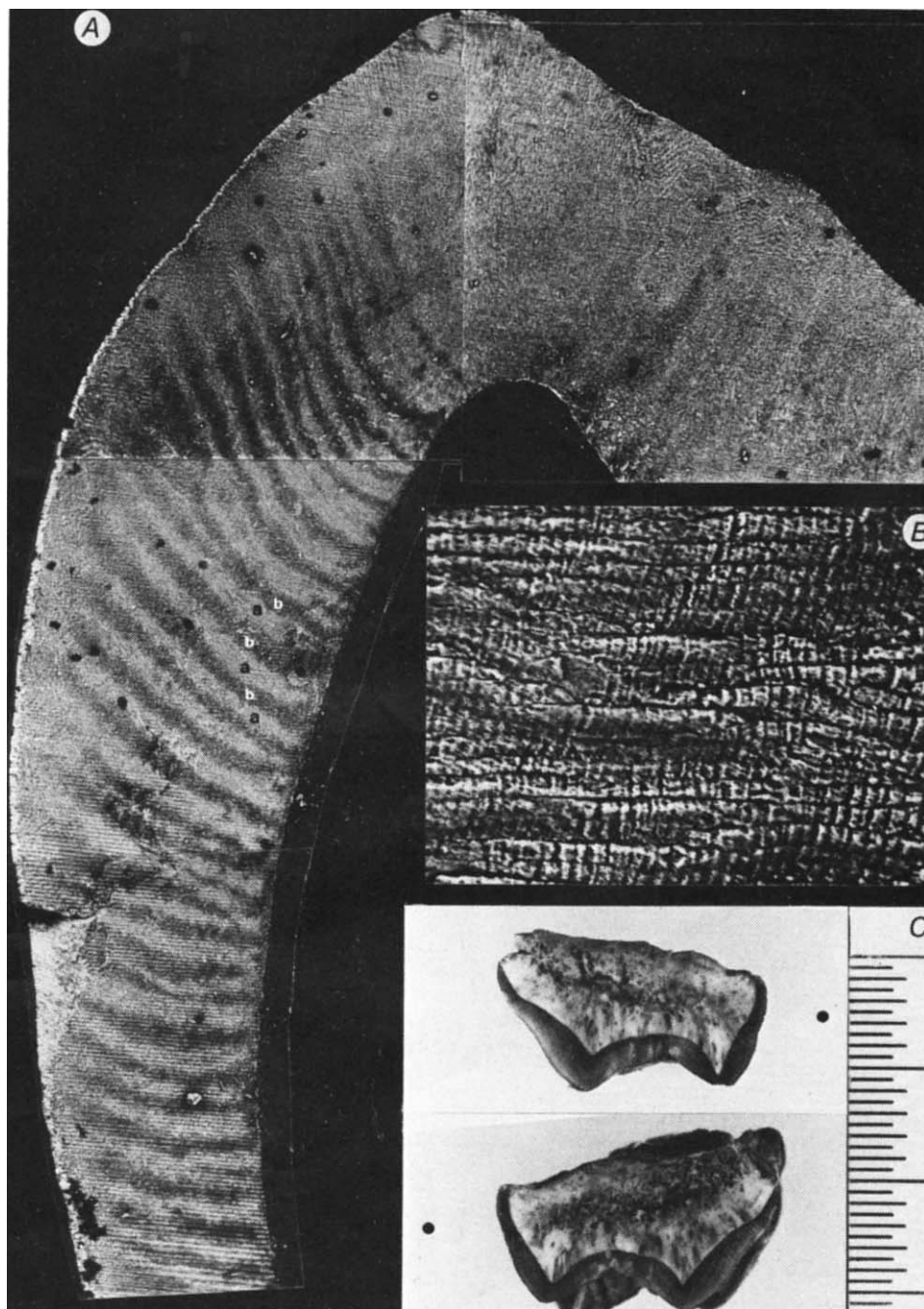
The distributions of these features were correlated in hominoid enamel. In all cases, pattern 3 enamel was associated with a cross-striation repeat interval of 5–7 μm (Fig. 2B), corresponding to the daily rate of ameloblast secretion (values are averages for large numbers of repeat intervals)²⁰. Hunter-Schreger bands occurred only in areas of pattern 3 enamel (Fig. 2A). Where pattern 1 enamel was found, it was associated with cross-striation repeat intervals of <2.5 μm , a much lower accretionary rate, and an absence of Hunter-Schreger bands. This indicates that, in hominoids, pattern 3 enamel with prism decussation is formed about three times faster than is non-decussating pattern 1 enamel.

All of the hominoid species examined had two very thin (20–30 μm) layers of slowly formed (pattern 1) enamel, one at the tooth surface and the other immediately adjacent to the enamel/dentine junction, corresponding to the start and finish of enamel production by the ameloblasts. In *Homo*, *Hyllobates* and *Sivapithecus*, the rest of the enamel was fast-formed (pattern 3) decussating enamel (Fig. 2) accounting for 90–95% of the enamel thickness.

Gibbons have thin enamel because it forms for a relatively shorter period (in relation to size) than does enamel in species of the great ape and human clade, which have extended developmental periods for tooth enamel relative to size. If all of their enamel was fast-formed it would be thick, as indeed it is for man. In the great apes, the thin enamel and intermediate/thick enamel form over the same relative time period as in the thick-enamelled species, but their enamel is thinner because the superficial layer of slow-formed pattern 1 enamel is much thicker and the average daily incremental rate is reduced. In *Pongo*, the slowly formed layer represents ~20% of maximum enamel thickness (~200 μm laid down at 2.5 μm per day and a further 50 μm laid down at 1.8 μm per day). In *Pan* and *Gorilla*, the slowly formed layer represents ~40% of maximum enamel thickness (~400 μm in *Gorilla* and ~300 μm in *Pan*), and was formed throughout at <1.5 μm per day. Thin enamel is structurally different in gibbons and African apes, and therefore not homologous, and the slowing down of enamel secretion is similarly non-homologous between the orang-utan and the African apes²⁰.

Thin enamel is a developmentally heterogeneous category and is therefore subdivided into two: first, thin enamel, all of which is formed at the fast (pattern 3) rate (termed thin, fast), present in gibbons; and second, thin enamel, a substantial portion of which is formed at the slow (pattern 1) rate (termed thin, slowed), present in the African apes. The intermediate/thin and the intermediate/thick categories could also be developmentally heterogeneous and are similarly subdivided. Thick enamel has been found only in species in which all of the enamel was formed at the fast (pattern 3) rate, and is therefore unlikely to have been developmentally heterogeneous in hominoids (Fig. 3a). This study therefore provides seven relevant categories of

Fig. 2 Enamel thickness and structure in extant and extinct Hominoidea. **A**, Montage of the enamel of the lingual cusp of a lower second molar of *Sivapithecus alpani* (BP 13) from Pasalar, Turkey (~14 Myr BP), with the enamel/dentine junction to the right. The cut face was lightly polished with 1- μ m diamond and then etched with 0.5% H_3PO_4 for 30 s. Hunter-Schreger bands extend across most of the enamel thickness, even where it is thickest. Only the region above the tip of the dentine cusp has no prism decussation. Parazones (a) and diazones (b) are labelled and result in the Hunter-Schreger band pattern. Field height, 4.27 mm. **B**, Cross-striations in a slowly formed region of the outer lingual enamel in a *Pongo pygmaeus* upper second molar. Cervical is towards the top, tooth surface to the left. The specimen was prepared by diamond polishing the cut face to a 1- μ m finish and then etching it with EDTA pH 7.2 for 18 h to remove the smeared enamel and to reveal the enamel prism boundaries and cross-striations. The micrograph is a back-scattered electron image^{26,27} which produces atomic number contrast, that is, a density-dependent image, when topographic relief is low. Field width, 146 μ m. **C**, Coronal longitudinal section through the mesiodistal cusps of a maxillary first molar (M^1) of *S. sivalensis* (M 13365). The upper section shows the anterior face revealed by the saw cut and the lower section the posterior face (350 μ m apart); the buccal side of the tooth is indicated by a black spot. The scale bar is marked with 0.5-mm divisions. Some variation in any enamel thickness data will be the result of imperfect measurements and imperfect sectioning. Oblique sections act to increase the apparent enamel thickness²⁰, and this effect has been minimized here by taking minimum, and therefore more nearly radial, measurements (of enamel thickness) from the two cut faces in conjunction with maximum values for all dentine/pulp measurements²⁰. When the saw cut has passed through the maximum diameter of the dentine horns, minimum values from both faces are used for enamel dimensions and maxima for dentine/pulp. When the saw cut has missed the plane of the maximum diameter of the dentine cusps (as with this *S. sivalensis* specimen), one face will be closer to the ideal plane of measurement than will the other²⁰. The posterior face of M 13365 lies closest to the ideal plane of section, as it produces minimum values for all enamel thickness measurements and maximum values for all dentine/pulp measurements, and was used for measurement.



enamel thickness/formation for assessment in fossil species, in comparison with the two ('thick enamel' and 'thin enamel') used previously^{7,8,13-15,17-19,22}.

Figure 3b shows the conditions of enamel thickness and formation rates in the common ancestors of the various hominoid clades as interpreted from the data presented here. They provide the basis for the assessment of the relationships of *Sivapithecus* and *Dryopithecus* (see Fig. 3b legend).

Thick-enamelled, human-like teeth have previously been used to support the hominine status of *Ramapithecus*^{1,17,18} and more recently *Sivapithecus*⁴⁻⁸ and even *Pongo*⁹, but these features are interpreted here to be primitive within the great ape and human clade (Fig. 3b legend). Thick enamel, having been considered the most important character in the assessment of the affinities of Miocene hominoids, turns out to be the least diagnostic of

the enamel thickness categories and of no relevance to the taxonomic placement of *Sivapithecus* (including '*Ramapithecus*') within the great ape and human clade²⁰.

Thick pattern 3 enamel does not define a hominid. Moreover, the common ancestor of the great apes and man, and of the African apes and man, would have had teeth resembling those of hominids. This should be considered when attempting to establish the earliest record of hominids from deposits of later Miocene to early Pliocene age. Of the living members of the great ape and human clade, only *Homo sapiens* retains the condition of enamel thickness and development from the common ancestor of that clade (Fig. 3b) and may therefore be regarded as the most dentally primitive member of it.

This work was carried out in the Department of Palaeontology, British Museum (Natural History) and the Departments

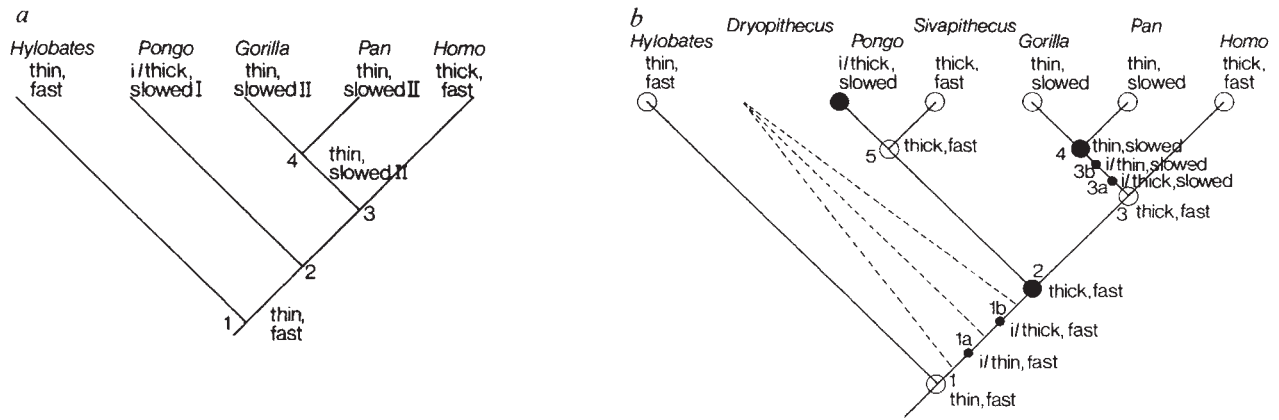


Fig. 3 Interpretations of the evolution of hominoid enamel. *a*, Enamel thickness and formation rates in extant great apes and humans; *b*, positions of *Sivapithecus* and *Dryopithecus*. The relationships among the living members of the great ape and human clade are based on cladistic interpretation of overall morphological pattern^{3-8,10-20,22,28,29}. The recently proposed close relationship between human and orang-utan⁹ depends very largely on the interpretation of thickened enamel as a shared derived character between these two genera, but as this has not been shown to be the case, and as our main aim here is to determine ancestral conditions, this view is not accepted here. *a*, Thin enamel predominates among cercopithecoids, platyrrhines, tarsiers and strepsirrhines^{4,15,18} and in *Hylobates*, so that it is parsimonious to interpret the condition in the last common ancestor (LCA) of the Hominoidea as being with thin, fast-formed (pattern 3) enamel (node 1), with this condition being retained in extant gibbons. Similarly, because both *Pan* and *Gorilla* have thin slowed-down enamel, it is likely that they retained this condition from their common ancestor (node 4). The situation for the LCA of the great ape and human clade (node 2) and for other contained clades is less clear. There are seven possibilities for the condition of enamel thickness/development at node 2 (see text). On the grounds that: (1) the thin enamel in gibbons and African apes is not homologous; (2) the slowed-down enamel in the African apes and the orang-utan is not homologous; and (3) the slowed-down pattern 1 enamel is developmentally derived from pattern 3 type with a relatively long total developmental period, in hominoids, the most parsimonious explanation for the distribution of enamel thickness and development among extant great apes and humans is that of thick enamel all of which formed at the fast, pattern 3 rate in the LCA of the great ape and human clade. *b*, This interpretation has considerable implications for the placement of fossil hominoids based on enamel thickness and structure. *Sivapithecus* is shown according to facial morphology^{13,14,16,20,28,29}, and this position is in accordance with enamel thickness data. Fossil taxa that do not have thick enamel could belong to several positions in hominoid phylogeny. If any of these taxa should be found to have either intermediate/thin or intermediate/thick fast formed enamel, it could be placed as the sister group of the great ape and human clade (nodes 1a, 1b). *Dryopithecus* is shown placed according to its possession of other derived characters shared with the great ape and human clade^{20,29}. Its position could be more completely determined if it were known whether *Dryopithecus* had thin, intermediate/thin or intermediate/thick enamel. Closed circles at nodes indicate derived conditions with respect to the previous ancestral node; open circles indicate nodes at which the ancestral condition is retained primitively. According to this interpretation, the common ancestor of the Hominoidea (node 1) had thin enamel all of which formed at the fast, pattern 3 rate. The common ancestor for the great ape and human clade (node 2) had thick enamel, all of which was formed at the fast, pattern 3 rate. Nodes 1a and 1b represent hypothetical common ancestors at probable stages of enamel evolution, node 1a with intermediate/thin enamel and node 1b with intermediate/thick enamel, all of which formed at the fast, pattern 3 rate. No living hominoids display these conditions, but we might expect to find these derived conditions in fossil hominoid species from the middle or late Miocene (such as *Dryopithecus* and/or *Kenyapithecus*). Thick, fast-formed pattern 3 enamel is primitively retained at node 3, the common ancestor of the African ape and human clade, and node 5, the common ancestor of *Sivapithecus* and the orang-utan. This condition is modified in the *Pongo* portion of the clade, with a secondary reduction to intermediate/thick enamel. This stage should be detectable in fossil pongines from the later Miocene or Pliocene or Pleistocene. Thick, fast-formed enamel is also primitively retained in the exclusively hominine clade. The common ancestor of the African apes (node 4) had thin enamel, much of which formed at a reduced daily rate and which is therefore interpreted as being secondarily reduced. Nodes 3a and 3b represent hypothetical ancestors of the African apes at stages of enamel evolution through which they must have passed.

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1. Simons, E. L. *Postilla* **57**, 1-9 (1961).
2. Ciochon, R. L. & Corruccini, R. S. (eds) *New Interpretations of Ape and Human Ancestry* (Plenum, New York, 1983).
3. Ciochon, R. L. in *New Interpretations of Ape and Human Ancestry*, 783-843 (Plenum, New York, 1983).
4. Kay, R. F. *Am. J. phys. Anthropol.* **55**, 141-152 (1981).
5. Kay, R. F. *Proc. natn. Acad. Sci. U.S.A.* **79**, 209-212 (1982).
6. Kay, R. F. *Int. J. Primatol.* **3**, 113-173 (1982).
7. Kay, R. F. & Simons, E. L. in *New Interpretations of Ape and Human Ancestry*, 577-624 (Plenum, New York, 1983).
8. de Bonis, L. in *New Interpretations of Ape and Human Ancestry*, 625-649 (Plenum, New York, 1983).
9. Schwartz, J. H. *Nature* **308**, 501-505 (1984).
10. Zihlman, A. H. & Lowenstein, J. M. in *New Interpretations of Ape and Human Ancestry*, 677-694 (Plenum, New York, 1983).
11. Wolpoff, M. H. in *New Interpretations of Ape and Human Ancestry*, 651-676 (Plenum, New York, 1983).
12. Greenfield, L. O. in *New Interpretations of Ape and Human Ancestry*, 695-703 (Plenum, New York, 1983).

13. Andrews, P. & Tekkaya, I. *Palaeontology* **23**, 85-95 (1980).
14. Andrews, P. & Cronin, J. E. *Nature* **297**, 541-546 (1982).
15. Gantt, D. G. thesis, Washington Univ. (1977).
16. Ward, S. C. & Pilbeam, D. R. in *New Interpretations of Ape and Human Ancestry*, 211-238 (Plenum, New York, 1983).
17. Simons, E. L. & Pilbeam, D. R. in *The Functional and Evolutionary Biology of the Primates*, 36-62 (Aldine, Chicago, 1972).
18. Simons, E. L. *J. hum. Evol.* **5**, 511-528 (1976).
19. Gantt, D. G. in *New Interpretations of Ape and Human Ancestry*, 249-298 (Plenum, New York, 1983).
20. Martin, L. B., thesis, London Univ. (1983).
21. Beynon, A. D. & Wood, B. A. *J. dent. Res.* **63**, 505, abstr. 144 (1984).
22. Andrews, P. & Tobien, H. *Nature* **268**, 699-701 (1977).
23. Boyde, A., thesis, London Univ. (1964).
24. Gantt, D. G., Pilbeam, D. R. & Steward, G. P. *Science* **198**, 1155-1157 (1977).
25. Vrba, E. S. & Grine, F. E. *Science* **202**, 890-892 (1978).
26. Boyde, A. & Martin, L. *Anat. Embryol.* **165**, 193-212 (1982).
27. Boyde, A. & Jones, S. J. *Anat. Embryol.* **168**, 211-226 (1983).
28. Martin, L. & Andrews, P. *Cour. Forsch. Inst. Senckenberg* **67**, 25-40 (1984).
29. Martin, L. & Andrews, P. *Primate Eye* **18**, 4-7 (1982).
30. Boyde, A. & Martin, L. in *Tooth Enamel* Vol. 4, 417-421 (Elsevier, Amsterdam, 1984).

Atrial natriuretic factor— a circulating hormone stimulated by volume loading

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The cardiocytes of mammalian cardiac atria contain granules very similar to those in endocrine cells^{1,2}. The number of these atrial granules is related directly to salt loading and blood volume³. Furthermore, crude extracts of rat atria and granule preparations have powerful natriuretic and diuretic effects^{4,5}. These effects are mediated by peptides identified previously as atrial natriuretic factor (ANF). The peptides are derived from a common precursor, whose structure has been elucidated recently⁶⁻¹⁵. Although there is indirect evidence from morphological studies that at least some of these peptides may be released into the blood and function as hormones, their presence in the blood has not yet been demonstrated. Here we describe a sensitive and specific radioimmunoassay for ANF and its stimulation on volume loading.

The rat synthetic peptides used here were the 21-amino acid atriopeptin I (AP I), the 23-amino acid atriopeptin II (AP II) and the 24-amino acid atriopeptin III (AP III)⁸. To obtain antibodies, New Zealand White rabbits were injected with AP II coupled to bovine thyroglobulin. AP III, which corresponds to the sequence of AP II extended at the C-terminus by a tyrosine residue, was iodinated with ¹²⁵I as a tracer. The radioimmunoassay for ANF was performed at a final antibody dilution of 1:80,000 and AP III was used for the construction of standard curves. All concentrations of ANF-like immunoreactive material were expressed as weight equivalents of AP III (see Fig. 2 legend).

When extracts from rat atria prepared as described in Fig. 1 legend were examined by radioimmunoassay, the mean concentration of ANF-like immunoreactive material was 86.9 ± 14.3 ng mg⁻¹ wet weight. Serial dilution of the extracts inhibited the binding of tracer to anti-ANF antiserum in parallel with the standard curve of ANF III.

Analysis of atrial extracts by HPLC revealed several immunoreactive peaks (Fig. 1a). Small amounts of ANF-like material were detected in the fractions where the synthetic standards eluted, but neither of the two major peaks corresponded to the position of these standards. Most of the immunoreactive material eluted with the upper third of the gradient, indicating a higher lipophilicity. The major peak appeared with a retention time of 54 min. Subsequent molecular mass sieving studies (not shown) indicated that this material represents higher relative molecular mass (*M_r*), consistent with the idea that it may represent precursor peptides. We studied the release of ANF-like material from rat atria *in vitro* using a modification of the isolated perfused heart preparation¹⁶. The heart was perfused with constant pressure through the aorta and buffer added to the right atrium via the upper vena cava. Increasing the perfusion rate gave a rise in right atrial pressure, monitored by a pressure transducer connected to a cannula in the lower vena cava. When right atrial pressure was raised by 1 mm Hg, the rate of ANF-like material release into the perfusate was doubled. A several-fold increase was observed after elevation of the pressure by 5 mm Hg (Table 1).

Figure 1b shows a HPLC-radioimmunoassay analysis of the perfusate collected at 5 mm Hg right atrial pressure. In contrast to the distribution of ANF-like material on HPLC of atrial extracts, the major fraction eluted with a retention time between that of AP II and AP III, whereas little high-*M_r* material was present. Muscle stretch is therefore a strong stimulus for liber-

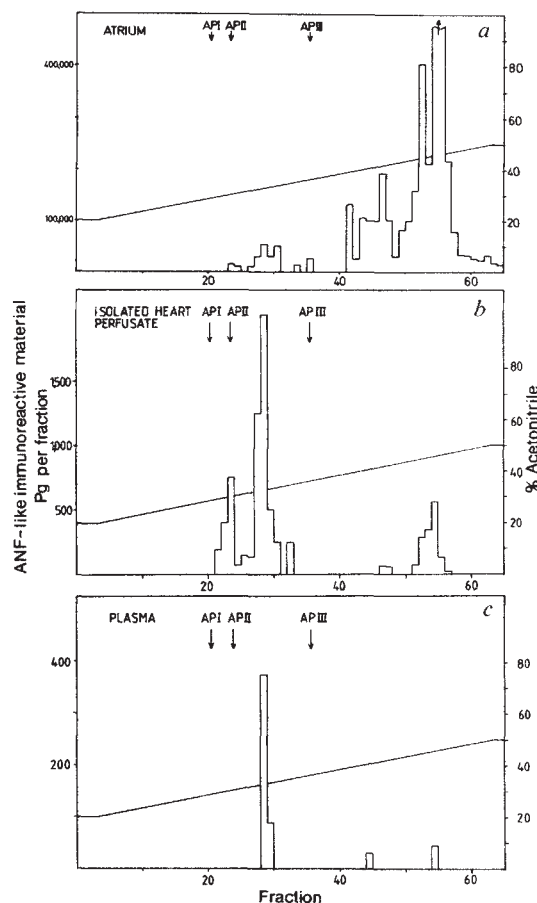


Fig. 1 Characterization of ANF-like immunoreactive material in: *a*, atrial tissue extracts; *b*, perfusate from isolated perfused rat heart; *c*, plasma, by reverse-phase HPLC using a Waters 2 Pump system and BondaPak C 18 column (40 cm × 1 cm). A linear gradient elution system was used from 20 to 50% over 60 min; solvent A, 0.01 M NH₄-trifluoroacetic acid (TFA) in water; solvent B, 0.01 M NH₄-TFA in acetonitrile; flow rate 1.0 ml min⁻¹. Fractions (1.0 ml) were collected, lyophilized and assayed for ANF-like material by radioimmunoassay (see Fig. 2 legend). Arrows indicate the elution positions of the synthetic peptides API, APII and APIII, respectively. The recovery of these peptides was between 85 and 90% as determined by radioimmunoassay.

Methods. *a*, Separation of extracts from rat atria. For extraction the tissue was minced and boiled for 15 min in 0.1 M HCl to avoid proteolysis. The cooled tissue was homogenized subsequently using a Polytron tissue homogenizer and centrifuged for 30 min at 3,000 g. The resulting supernatant was extracted using ODS-silica cartridges as described for plasma in Fig. 2 legend, and subjected to HPLC. *b*, Analysis of ANF-like material in the perfusion buffer from the isolated perfused rat heart (see Table 1). Perfusate was collected during pressure stimulation of the right atria (5 mm Hg) and extracted by using ODS-silica cartridges before application to the HPLC column. *c*, Characterization of rat plasma ANF-like material. After insertion of chronic catheters into the femoral vein, rats were anaesthetized with pentobarbital and loaded with 8 ml normal saline as described in Fig. 2 legend. Blood was withdrawn from the aorta after laparotomy, centrifuged and plasma-extracted by passing through ODS-silica cartridges according to the procedure in Fig. 2 legend. The material subjected to HPLC corresponded to 2 ml plasma. Note that the retention time of the peak fraction is identical to that from the perfusate in *b*.

ation of ANF-like immunoreactive material from atria; small ANF peptides rather than precursor molecules are released.

ANF-like material was also found in rat plasma. When extracted according to the procedure described in Fig. 2 legend, the mean plasma concentration in barbiturate-anaesthetized rats was 54.6 ± 13.9 pg ml⁻¹ (mean $\pm$ s.d., *n* = 10). Plasma levels showed marked and dose-dependent increases on volume expansion (Fig. 2). Infusion of 2 ml normal saline produced a

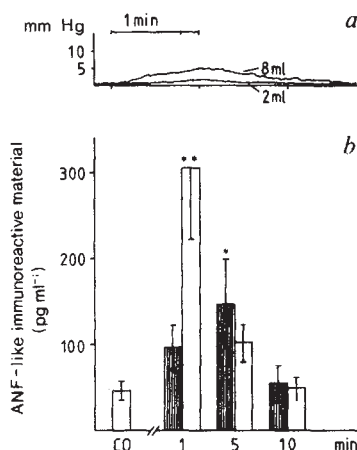


Fig. 2 Influence of volume expansion on ANF-like material in rat plasma. Values are expressed as weight equivalents of AP III (mean $\pm$ s.d., * P < 0.05; statistical analysis by Bonferroni test). Seventy male Wistar rats (370–420 g) in which catheters had been inserted in the femoral vein, were divided into seven groups of 10 animals. Three groups received either a 2-ml (*b*, shaded columns) or 8-ml (*b*, open columns) saline infusion i.v. under pentobarbital anaesthesia. The remaining group served as a control. Infusion time 1 min. Changes in right atrial pressure during volume load were measured in four animals by means of a catheter inserted into the right atrium through the jugular vein. *a*, Two representative traces.

Methods. Preparation of blood samples: Following laparotomy, blood was taken from the aorta either 1, 5, or 10 min after termination of volume load into plastic syringes containing aprotinin (1,000 kallikrein inactivator units ml^{-1}), soybean trypsin inhibitor (100 α -N-benzoyl-L-arginine ethyl ester units ml^{-1}) and EDTA (1 mg ml^{-1}). Plasma was separated by centrifugation and processed for extraction of ANF-like material as follows: 2 ml of each sample were diluted with 3 vol of 4% acetic acid and after centrifugation pumped at a rate of 4 ml min^{-1} through a small column containing ODS particles (Porasil; Machrey and Nagel). After a wash with 5 ml water, the absorbed peptides were eluted with 86% ethanol in 4% acetic acid. After evaporation and lyophilization, the dry residue was dissolved in radioimmunoassay buffer for determination of ANF-like material. Radioimmunoassay of ANF-like material: Synthetic rat AP II (Bachem) was used for immunization. The peptide was coupled to bovine thyroglobulin using carbodiimide²¹ and 100 μg of the conjugate in 1 ml saline were emulsified with the same volume of complete Freund's adjuvant and injected intradermally at multiple sites on the backs of NZW rabbits. The first four immunizations were given at weekly intervals; subsequent booster injections were made every month. Rat AP III was used for labelling. Iodination with ^{125}I was performed according to the chloramine-T method²². For separation of the labelled peptide from free iodine, the reaction mixture was passed through ODS-silica cartridges and eluted as described above including a wash with 10% ethanol in 4% acetic acid before the final elution step. The specific activity of the tracer was 80–100 $\mu\text{Ci } \mu\text{g}^{-1}$. The radioimmunoassay was performed in 0.1 M Tris buffer, pH 7.4, containing 0.1% gelatine. Synthetic AP III (1.95–1,000 pg per tube) was used to construct standard curves. The incubation mixture consisted of 0.2 ml buffer, 0.1 ml standard or sample and 0.1 ml antibody (final dilution 1:80,000). Tracer (4,000 c.p.m. per tube) was added after 48 h at 4 °C. After an overnight second incubation, separation of free from antibody-bound ^{125}I -peptide was achieved by adding 0.2 ml dextran-coated charcoal. Using this procedure, the lowest concentration of AP III yielding a binding significantly different from that in the absence of standard at the 95% confidence interval, was 2 pg per tube. The 50% intercept was at 41 pg per tube. The inter-assay variation was 14.1% ($n = 6$), and the intra-assay variation was 14.1% ($n = 6$), and the intra-assay variation 7.1% ($n = 10$). A complete cross-reaction was observed with AP I and AP II. The antibodies did not (<0.001%) cross-react with the following peptides: angiotensin I and II, corticotropin-releasing factor, adrenocorticotropin, oxytocin, vasopressin, β -endorphin, α -neo-endorphin, dynorphins of different chain lengths, enkephalins, neuropeptide Y, somatostatin, vasoactive intestinal polypeptide. For studies on the recovery of ANF from plasma, various dilutions of synthetic AP III were added to plasma treated with charcoal for elimination of endogenous peptides. Using the ODS cartridges procedure for extraction, the recovery of synthetic AP III ranged from 62 to 70% both in the upper and lower ranges of the standard curve. The serial dilutions of extracts from rat plasma as well as atrial tissue and heart perfusate inhibited the binding of tracer to antibody in parallel with the standard curve of AP III.

the peak values appeared at 6 min after infusion (data not shown).

For characterization of the ANF-like immunoreactive material in plasma, the blood volume of five rats was expanded by intravenous (i.v.) administration of 8 ml saline. The animals were bled 1 min after the infusion, the blood was pooled, the plasma extracted as described in Fig. 2 legend and subjected to HPLC. Radioimmunoassay revealed one major peak between the positions of AP II and AP III (Fig. 1c), corresponding exactly to the major peptide in the heart perfusate (Fig. 1b). In addition, very small amounts of ANF-like material were detected in the position of the high- M_r peptides of atrial extract. When the addition of aprotinin and soybean trypsin inhibitor to the blood was omitted, the peak fraction of the plasma HPLC was shifted to the position of AP III, indicating that degradation by plasma enzymes affects ANF metabolism.

This represents the first time that ANF has been demonstrated and characterized in the blood, although many attempts have been made to identify a natriuretic hormone that could increase renal excretion of salt and water^{17,18}. Material having biological natriuretic activity has been reported in the blood and urine of several species¹⁹ and its concentration was found to increase when the volume of blood or extracellular fluid was expanded experimentally^{17–20}. Our HPLC-radioimmunoassay data show for the first time that rat plasma contains a substance immunologically related to the natriuretic peptides in and released from rat atria^{6–15}. The plasma concentration is increased by physiological stimuli such as volume expansion.

Cardiac tissue contains many natriuretic peptides derived from the same precursor molecule. It is not yet known which of these are extraction artefacts and which occur naturally. In the plasma, we found a single peptide identical to the one released from the heart. Plasma ANF is closely related to but not identical with API, APII and APIII, provided there is adequate protection from enzymatic degradation, in accordance with the suggestion that these peptide fragments would not occur in the heart if proteolysis was prevented during the extraction procedure from the atria¹⁰. Elucidation of the nature of circulating ANF will be possible when more ANF peptides have been synthesized and are available as reference substances. The knowledge of the exact amino-acid sequence is probably not crucial, as the peptides so far described usually exhibit variations in length of the amino-terminal region, which seem not to affect their biological function.

The elution pattern of ANF-like immunoreactive material obtained from atrial extracts indicates that most of it is not identical to the plasma peptide, but probably represents storage

Table 1 Effect of mean right atrial pressure on the rate of release of ANF-like immunoreactive material from the isolated perfused rat heart (pg min^{-1})

Expt no.	Change in right atrial pressure		
	Control	1 mm Hg	5 mm Hg
1	768	972	—
2	575	740	—
3	533	883	—
4	585	—	1,763
5	345	—	1,250
6	408	—	845

Rat hearts were removed and perfused through the aorta with Krebs/Ringer's solution at a constant pressure (122 mm Hg). The upper vena cava was cannulated and buffer infused into the right atrium at various rates to increase the mean right atrial pressure by either 1 or 5 mm Hg. The atrial pressure was monitored continuously by a pressure transducer connected to a catheter inserted into the lower vena cava. Mean atrial pressure varied between -0.5 and $+0.2$ mm Hg under basal conditions. Perfusate was collected and 1-min fractions extracted using the octadecasilyl (ODS)-silica cartridges technique for the radio-immunological determination of ANF-like immunoreactive material. The 5 mm Hg perfusate was used for HPLC characterization (see Fig. 1b).

two- to threefold rise in plasma ANF-like material. Administration of 8 ml, corresponding to a $\sim 30\%$ volume expansion, gave a sixfold increase. The peaks in right atrial pressure in these experiments were 1–1.5 and 5 mm Hg, respectively. Similar results were obtained in experiments using whole blood obtained from Wistar rats of the same age and sex. In these conditions

forms of ANF serving as biosynthetic precursors for the ANF released into the blood. Our data demonstrate that, on stimulation, small concentrations of this precursor peptide are also released into the blood. Whether the conversion to the hormonal plasma peptide occurs mainly in the heart, lung or plasma remains to be elucidated. Our experiments on the isolated perfused heart suggest that most of the plasma ANF is formed in cardiac tissue, similar to other hormones.

The release of ANF-like immunoreactive material in response

to volume load is rapid. We find an increase of plasma concentration as soon as 1 min after stimulation. The exact mechanism involved in the mediation of release is unclear. As shown here, the stretch of atrial cardiocytes produced by volume expansion may induce secretion of the natriuretic and diuretic peptides. Whether, in addition, higher centres in the brain are involved via neuronal reflex arcs will be the subject of future experiments.

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- Kisch, B. *Expl. Med. Surg.* **14**, 99-112 (1956).
- Palade, G. E. *Anat. Rec.* **139**, 262 (1961).
- Marie, J. P., Guillemot, H. & Hatt, P. Y. *Path. Biol.* **24**, 549-554 (1976).
- Sonnenberg, H., Veress, A. T., Borenstein, H. B. & de Bold, A. J. *Physiologist* **23**, 13 (1980).
- Garcia, R., Cantin, M., Thibault, G. & Genest, J. *Experientia* **38**, 1071-1073 (1982).
- Atlas, S. A. *et al. Nature* **309**, 717-719 (1984).
- Flynn, T. G., de Bold, M. L. & de Bold, A. J. *Biochem. biophys. Res. Commun.* **117**, 859-865 (1983).
- Geller, D. M. *et al. Biochem. biophys. Res. Commun.* **120**, 333-338 (1984).
- Kangawa, K., Fukuda, A., Minamino, N. & Matsuo, H. *Biochem. biophys. Res. Commun.* **119**, 933-940 (1984).
- Kangawa, K., Fukuda, A., Kubota, I., Hayashi, Y. & Matsuo, H. *Biochem. biophys. Res. Commun.* **121**, 585-591 (1984).
- Maki, M. *et al. Nature* **309**, 722-724 (1984).
- Oikawa, S. *et al. Nature* **309**, 724-726 (1984).
- Seidah, N. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 2640-2544 (1984).
- Seidmann, C. E. *et al. Science* **225**, 324-326 (1984).
- Yamanaka, M. *et al. Nature* **309**, 719-722 (1984).
- Langendorf, O. *Pflügers Arch. ges. Physiol.* **61**, 291-332 (1985).
- Haddy, F. J. *Ann. intern. Med.* **93**, 781-784 (1983).
- de Wardener, H. E. *Clin. Sci.* **21**, 249-258 (1961).
- de Wardener, H. E. *Clin. Sci. molec. Med.* **53**, 1-8 (1977).
- Sealey, J. E., Kirschmann, J. D. & Laragh, J. H. *J. clin. Invest.* **48**, 2210-2224 (1969).
- Skowsky, W. R. & Fisher, D. F. *J. Lab. clin. Med.* **80**, 134-144 (1972).
- Hunter, H. W. & Greenwood, F. C. *Nature* **194**, 495-496 (1984).

Homogeneous interferon-inducing 22K factor is related to endogenous pyrogen and interleukin-1

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In vitro stimulation of mononuclear cells from human peripheral blood with mitogens causes the release of factors (monokines and lymphokines) which possess distinct biological activities. One such factor, termed 22K, can induce production of human β -interferon (HuIFN- β) in cultured human fibroblasts, thereby rendering these cells resistant to virus infection¹⁻³. Here we report the complete purification and partial sequencing (39 N-terminal amino acids) of this factor, whose relative molecular mass was estimated by SDS-polyacrylamide gel electrophoresis to be 17,000 (17K). In addition to an antiviral effect, the pure protein exhibits several other biological activities. Most significantly, intravenous (i.v.) injection of the factor in rabbits caused fever and granulopenia at doses of 0.1-1 μ g per kg, effects which we attribute to a monokine called endogenous pyrogen (EP). *In vitro*, the protein was scored as positive in a LAF (lymphocyte-activating factor) assay at 0.1-1 ng ml⁻¹. LAF and EP are considered to be members of one family of monokines, called interleukin-1 (IL-1)⁴. For this reason, and also because the amino-acid sequence of the 22K factor is at least partially homologous to a complementary DNA-derived IL-1 sequence, we postulate that the 22K factor also belongs to the IL-1 family.

Electrophoretically pure 22K was obtained from crude human lymphokine preparations by a four-step purification schedule summarized in Fig. 1 legend. The protein migrated on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as a single Coomassie blue-stained band with a relative molecular mass (M_r) of 17,000. A parallel unstained gel was sliced, each slice eluted and the eluates tested for antiviral activity. The antiviral activity coincided with the single stained band. We also determined the N-terminal amino-acid sequence of the protein using a gas-phase sequenator. Figure 2 shows the sequence of 39

N-terminal amino acids obtained from three runs, each using ~15 μ g of protein. At most of the 39 positions, only a single residue was found in each of the three runs, indicating that the 22K-containing fractions obtained by fast protein liquid chromatography (FPLC) had a high degree of purity. At several positions two or three different residues were seen, suggesting that the factor is microheterogeneous. This microheterogeneity may reflect allelic polymorphism or the existence of a multi-gene family.

Although originally discovered by virtue of its IFN- β -like antiviral effect², 22K may have biological significance not related to the interferon system. In fact, the interferon-like antiviral effect of 22K is restricted in several ways: only certain diploid cell strains or continuous cell lines are sensitive and these may lose sensitivity on passage³; inhibition of the cytopathic effect is often incomplete, even with high doses of 22K; the reduction of virus yield generally does not exceed 3-10-fold; induction of HuIFN- β and its mRNA is detectable only when 22K is aided by cycloheximide or a combination of cycloheximide and actinomycin D⁵.

Several experiments were done to determine whether 22K has biological activities previously assigned to 'factors' released by mononuclear cells; Table 1 summarizes the results. Pure 22K preparations did not inhibit the growth of cells on which they exerted an antiviral effect, nor did the factor show any activity in standard assay systems for lymphotoxin⁶, interleukin-2 (IL-2)⁷ and natural killer (NK) cell stimulation⁸, while the LAF test for IL-1 (ref. 9) was positive. 22K preparations, including those consisting of electrophoretically homogeneous material, were able to replace crude lymphocyte supernatants as granulocyte-macrophage colony-stimulating factor (GM-CSF) in human bone marrow cultures¹⁰, suggesting that 22K may cause the release of such factors from a subpopulation of bone marrow cells.

Strong inflammatory responses were seen when partially purified 22K preparations were injected intracutaneously in man, monkey or rabbit. Macroscopically, the reaction consisted of mild swelling and redness, maximal at 16 h post-injection and subsiding after 36-48 h. Microscopic examination of rabbit skin biopsies, taken 24 h after injection, revealed infiltration by polymorphonuclear cells, suggesting a direct or indirect chemotactic effect of the injected material. When fractions from gel filtration runs were tested in rabbits, the peak of skin reactivity coincided with that of antiviral activity. At this stage of purification, 22K may be presumed to still contain bioactive impurities, for example, IL-2, some of which may be responsible for the skin reactivity. However, on FPLC, skin reactivity also co-eluted with the electrophoretically pure antiviral factor.

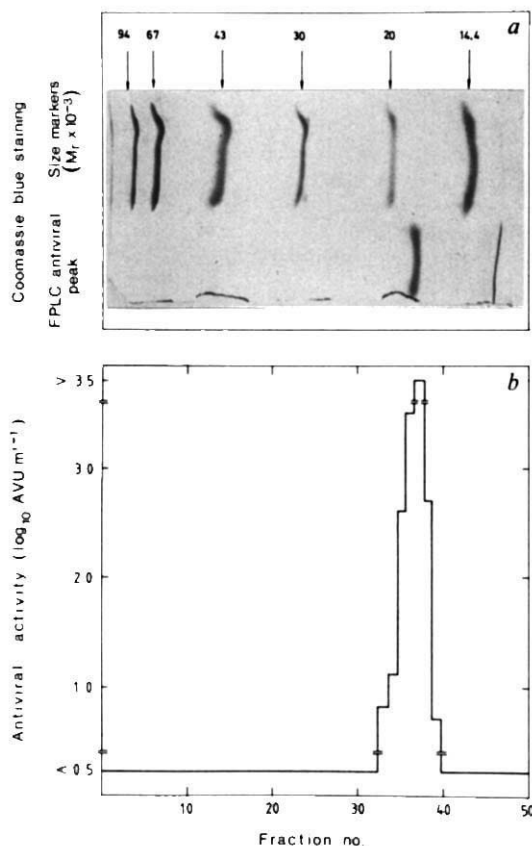


Fig. 1 SDS-PAGE of a pure preparation of 22K protein. Crude lymphokine/monokine preparations from Concanavalin A-induced mononuclear cell suspensions were concentrated and partially purified by adsorption to silicic acid and elution with 50% ethylene glycol containing phosphate-buffered saline (PBS) at pH 7.4 or with glycine buffer at pH 2.0 as described previously². These eluates had an average specific activity of 10^4 AVU per mg protein; 1 AVU ml^{-1} is defined as the minimal concentration required to inhibit the cytopathic effect of vesicular stomatitis virus on sensitive human MG-63 cells, using a regular interferon microtitre assay system². These preparations contained several lymphokines, including γ -interferon (IFN- γ) and IL-2 (results not shown). The second purification step consisted of adsorption of impurities to DEAE-Sephacel in 20 mM Tris-HCl buffer, pH 8.0. The DEAE-Sephacel-adsorbed fraction (specific activity $10^{5.1}$ AVU mg^{-1}) was concentrated further and fractionated by gel filtration on Ultrogel AcA 54. Antiviral activity eluted in fractions corresponding to a M_r of 45,000 (fractions characterizable as IFN- γ) and 22,000 (fractions characterizable as IFN- β -like antiviral protein). The 22K fractions had a specific activity of $\geq 10^{6.5}$ AVU mg^{-1} ; they were free of IFN- γ ($M_r \sim 45,000$), but were contaminated with IL-2 ($M_r \sim 25,000$). The 22K peak fraction from gel filtration was finally purified to homogeneity by FPLC using a Mono S cation-exchange column (Pharmacia Fine Chemicals); the activity was applied in 50 mM formate buffer, pH 4.0. The Mono S column was then subjected to a linear NaCl gradient (0–0.65 M) at a flow rate of 1 ml min^{-1} . The antiviral activity invariably eluted as a single peak at 0.37 M NaCl. The specific activity was increased to $\geq 10^{7.0}$ AVU mg^{-1} . Fractions with high biological activity were pooled and analysed by SDS-PAGE on a linear 12% gel in reducing conditions. Coomassie blue-stained protein bands are shown in **a**. A parallel unstained gel was sliced and each slice was eluted with PBS; the eluates were stabilized with human serum albumin and dialysed against PBS to localize the antiviral activity (in **b**). For several experiments there was good quantitative agreement between the presence of the stained band and antiviral activity. Gels loaded with too little 22K failed to show the stained band and all fractions scored negatively. The protein content of antivirally active fractions, obtained at different purification steps, was measured by a fluorimetric assay, as described². For fractions also containing Tris buffer protein, determinations were done using a Coomassie brilliant blue G-250 binding assay (Bio-Rad). In both cases bovine serum albumin was used as a standard.

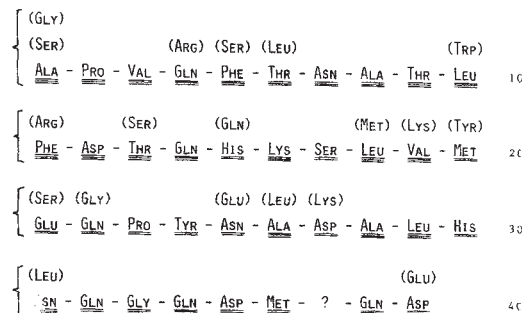


Fig. 2 N-terminal amino-acid sequence of the 22K factor. The number of lines beneath each residue indicates the number of runs in which the amino acid was observed. Brackets indicate detection of other residues.

Methods. Aliquots ($\sim 15 \mu\text{g}$) of protein obtained from three FPLC runs (see Fig. 1 legend) were sequenced in a gas-phase sequencer¹⁵ (Applied Biosystems, model 470A). The samples were not carboxy-methylated before sequence analysis, so that cysteine residues could not be detected. The phenylthiohydantoin (PTH) amino acids obtained from the sequencer were identified by HPLC on an IBM cyanopropyl column ($0.46 \times 25 \text{ cm}$) using a separation procedure similar to that described by Hunkapiller and Hood¹⁶. The equipment used was the Waters Intellink system supplemented with a Perkin Elmer LC75 and a Pye Unicam LCUV detector set at 269 and 323 nm respectively.

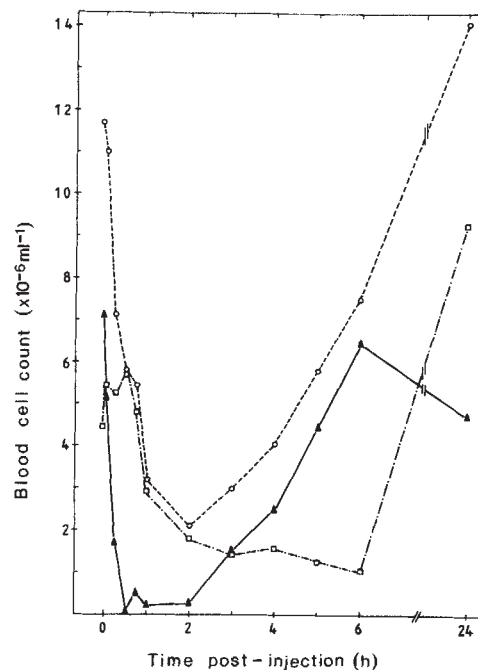


Fig. 3 Effect of i.v. injection of 22K protein on peripheral leukocyte counts in rabbits: $\circ$ — $\circ$, leukocytes (total); $\square$ — $\square$, lymphocytes; $\blacktriangle$ — $\blacktriangle$, granulocytes. The material injected was a FPLC-purified preparation (see Fig. 1 legend) containing $\sim 1 \mu\text{g}$ of protein and $10^{4.3}$ AVU. Rabbits injected with saline or heat-treated (90°C , 30 min) 22K factor did not show alterations in total or differential leukocyte counts.

Pure preparations of 22K were injected intravenously (i.v.) in rabbits to study the possible acute toxicity. Depending on the dose, increases in rectal temperature of up to 1.5°C were noted; this effect was not due to contamination by bacterial endotoxins. Indeed, the injected 22K preparations lost their pyrogenicity after heat treatment (90°C for 30 min), while the pyrogenicity of an *Escherichia coli* lipopolysaccharide preparation was heat-resistant. Pure preparations were also free of lipopolysaccharide as tested by the *Limulus* amoebocyte lysate assay ($\leq 0.6 \text{ ng}$ lipopolysaccharide in the highest injected dose, 3,000 antiviral units (AVU) per kg). Figure 3 shows that i.v. injection of pure 22K in rabbits caused a virtually immediate, but reversible, leukopenia. Granulocytes disappeared from the

Table 1 Biological properties of homogeneous 22K factor

Biological activity	Test system	Result (minimum effective dose or highest dose tested)
<i>In vitro</i>		
Antiviral effect	Inhibition of cytopathic effect in human diploid cells and MG-63 cells	Positive (1)*
CSF	Granulocyte-macrophage colony formation in human bone marrow cultures	Positive (1-3)*
IL-1	³ H-thymidine incorporation by human thymocytes	Positive (1)*
IL-2	³ H-thymidine incorporation by IL-2-dependent cytotoxic T-lymphocyte line	Negative (>500)*
NK-cell stimulation	Increase in natural cytotoxicity of human peripheral blood mononuclear cells against K-562 cells (⁵¹ Cr-release)	Negative (>100)*
Lymphotoxin	Cytolysis of mouse L-929 cells (subline α) pretreated with mitomycin C	Negative (>250)*
Cell growth	³ H-thymidine incorporation in log-phase cultures (human MG-63 and HEP-2)	Negative (>100)*
	Viable cell counts in log-phase cultures (human MG-63, HEP-2, U-937, HL-60 and diploid cells)	Negative (>100)*
<i>In vivo</i>		
Skin reactivity	Local inflammation after intracutaneous injection in rabbits	Positive (10,000)†
Pyrogenicity	0.5 °C Increase in rectal temperature after i.v. injection (3, 100 and 3,000 AVU per kg)	Positive (100 per kg)†
Leukopenia	Total and differential leukocyte counts after i.v. injection in rabbits (100, 1,000 and 10,000 AVU per kg)	Positive (1,000 per kg)

* Antiviral units (AVU) ml⁻¹ as measured on MG-63 cells; † AVU.

circulating blood within 30 min after injection; lymphocyte counts decreased from 5,000 to a minimum of 2,000 cells per mm³ in 2 h. Granulocytes reappeared from 2 h onwards and reached normal counts after 6 h; lymphocyte counts took 24 h to return to normal. The new cells were morphologically normal. Erythrocyte counts remained unaffected. The mechanism of this leukopenia is unclear, but present knowledge suggests a temporary adherence of the cells to the endothelia of the blood vessels and/or lymphoreticular organ system; however, other explanations are possible.

The *in vitro* antiviral effect of 22K occurred at doses of the order of 0.1–1 ng ml⁻¹, the systemic *in vivo* effect (leukopenia and pyrogenicity) at doses of 0.1–1 µg per kg. The molecule can thus be counted among the pharmacologically very active substances, such as lymphokines, interferons, neuropeptides, etc. Therefore, it seems reasonable to assume that 22K is a cytokine; the fact that it is released after mitogenic stimulation of leukocytes suggests that lymphocytes or monocytes are the producing cells and that 22K is generated in the body at sites where cellular immune reactions occur (for example, delayed-type hypersensitivity reactions, transplant rejection sites, etc.).

The strong pyrogenicity and haematological effects of 22K are both reminiscent of endotoxin-induced reactions¹¹, and suggest that 22K is related or identical to endogenous pyrogen. This monokine is considered to be related to IL-1 and to be involved in granulopoiesis¹², probably by stimulating production of CSFs. The conclusion that the 22K factor is essentially (an) endogenous pyrogen is therefore consistent with the fact that it has activity in a LAF assay and that it can replace CSF preparations in bone marrow cultures. The antiviral effect, which apparently is due to activation of the HuIFN- β system may, for reasons already mentioned, be of lesser physiological importance.

After submission of this paper, there have been reports on the isolation and sequencing of cDNA clones of human and murine IL-1 (refs 13, 14); these predict IL-1 gene products of M_r 31,000, which probably are 'precursor' proteins of the much smaller natural IL-1 products. The N-terminal amino-acid sequence of our 22K factor shows extensive homology with the middle part of the cDNA-derived human IL-1 precursor sequence. Alignment of the two sequences allows us to unambiguously define the position of the N-terminal amino acid of the biologically active human IL-1 in the precursor sequence determined by Auron *et al.*¹³. This provides further evidence that our 22K factor must be considered to be IL-1. Moreover, the fact that the homology was incomplete, together with the observation of multiple residues at several positions in the sequence, supports the notion that IL-1 comprises a micro-heterogeneous family of molecules.

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- De Ley, M. *et al.* *Eur. J. Immun.* **10**, 877–883 (1980).
- Van Damme, J. *et al.* *Eur. J. Immun.* **11**, 937–942 (1981).
- Van Damme, J., Billiau, A., De Ley, M. & De Somer, P. *J. gen. Virol.* **64**, 1819–1822 (1983).
- Dinarelli, C. A. *Rev. infect. Dis.* **6**, 51–95 (1984).
- Van Damme, J. *et al.* *J. gen. Virol.* (in the press).
- Spofford, B. T., Daynes, R. A. & Granger, G. A. *J. Immun.* **112**, 2111–2116 (1974).
- Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. *J. Immun.* **120**, 2027–2032 (1978).
- Claeys, H., Van Damme, J., De Ley, M., Vermynen, C. & Billiau, A. *Br. J. Haemat.* **50**, 85–94 (1982).
- Maizel, A. L., Metha, S. R., Ford, R. J. & Lachman, L. B. *J. exp. Med.* **153**, 470–475 (1981).
- Iscoe, N. N., Senn, J. S., Till, J. E. & McCulloch, E. A. *Blood* **37**, 1–5 (1971).
- Elin, R. J. & Wolff, S. M. A. *Rev. Med.* **27**, 127–141 (1976).
- Kampschmidt, R. F. & Upchurch, H. F. *Proc. Soc. exp. Biol. Med.* **155**, 89–93 (1977).
- Auron, P. E. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **81**, 7907–7911 (1984).
- Lomedico, P. T. *et al.* *Nature* **312**, 458–462 (1984).
- Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. *J. biol. Chem.* **256**, 7990–7997 (1981).
- Hunkapiller, M. W. & Hood, L. E. *Meth. Enzym.* **91**, 486–493 (1983).

A hapten-specific chimaeric IgE antibody with human physiological effector function

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Immunoglobulin E (IgE; see ref. 1) has a central role in allergic reactions although it rarely exceeds 5 µg ml⁻¹ even in the serum of severely allergic individuals. Both mast cells and basophils possess receptors which bind the Fc portion of IgE with high affinity; crosslinking of membrane-bound IgE by allergen results in degranulation of the cell and release of a variety of pharmacologically active mediators including histamine. Myeloma IgE

has been successfully used to block the skin sensitizing activity of allergic sera²; however, human myeloma IgE is clearly in limited supply. The emergence of techniques allowing the stable introduction of immunoglobulin gene DNA into myeloma cells³⁻⁵ has allowed us to construct a mouse cell line that secretes a chimaeric IgE, $\lambda 1$ antibody whose heavy chain is composed of a human C_{ϵ} constant region fused to a mouse variable (V_H) region. This chimaeric IgE is specific for the hapten 4-hydroxy-3-nitrophenacetyl (NP) and can, when crosslinked by antigen, trigger the degranulation of human basophils. When not crosslinked, however, the chimaeric IgE can prevent the passive sensitization of these cells by sera from allergic subjects.

The mouse plasmacytoma J558L secretes $\lambda 1$ light chains but does not produce an immunoglobulin heavy chain³. The strategy used in the production of the recombinant IgE antibody was to introduce a plasmid that encodes a complete immunoglobulin ϵ heavy chain into J558L and thus direct the production of an IgE, $\lambda 1$ antibody. The plasmid used for this purpose, pSV- $V_{NP}H_{\epsilon}$ (see Fig. 1), consists of an immunoglobulin ϵ heavy-chain gene cloned into the vector pSV2gpt (ref. 6). The heavy chain consists of a mouse variable region and a human ϵ constant region. This heavy chain, in combination with the $\lambda 1$ light chain expressed by the J558L myeloma, should produce an antibody specific for the hapten NP. In plasmid pSV- $V_{NP}H_{\epsilon}$, the exons encoding the variable (V_H) and constant (C_H) regions of the heavy chain are separated by only 300 base pairs of intron; this contrasts with the normal *in vivo* situation where the two sets of exons are separated by an intron of ~8 kilobases (kb). The transcription enhancer element^{4,7,8}, which normally lies between V_H and C_H , is located upstream of the V_H exons in pSV- $V_{NP}H_{\epsilon}$; we have shown previously that the enhancer is active on the V_{NP} promoter at this position⁴.

pSV- $V_{NP}H_{\epsilon}$ was introduced into J558L cells by spheroplast fusion, and stably transfected clones were selected as described previously⁹. Transfectants were obtained with a frequency of between 10^{-3} and 10^{-4} in different experiments. Culture supernatants of these transfectants were assayed for production of $\lambda 1$ -bearing anti-NP antibody by radioimmunoassay; between 50 and 80% of the clones were positive. Antibody was purified from culture supernatants of several transfected clones by affinity chromatography on a hapten-Sephrose column, with a yield of ~2 mg of antibody per litre.

Binding inhibition assays were used to demonstrate that the purified antibody displayed human ϵ antigenic determinants (Fig. 2). The affinity-purified chimaeric antibody competes with the binding of radiolabelled human myeloma IgE to both monoclonal (Fig. 2a) and polyspecific (Fig. 2b) anti- ϵ antisera. Fur-

thermore, binding of radiolabelled myeloma IgE to the anti- ϵ antiserum was inhibited completely (Fig. 2b), indicating that the chimaeric antibody displays all the ϵ antigenic determinants recognized by this polyclonal antiserum. The chimaeric antibody competed with the binding of the radiolabelled myeloma IgE to the anti- ϵ antisera better than did the unlabelled myeloma

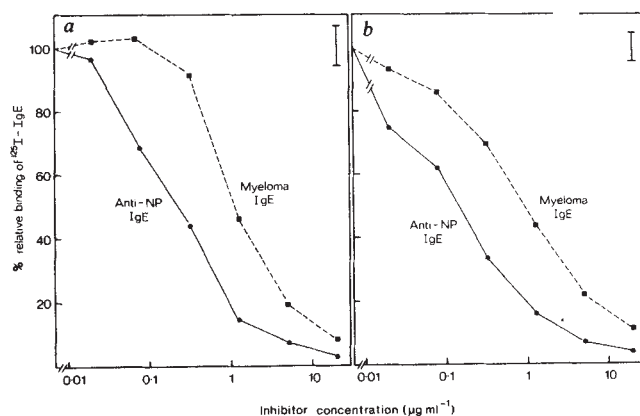


Fig. 2 Serological analysis of chimaeric IgE by binding inhibition assays. Assays were performed in which the binding of a radioiodinated human myeloma IgE to either a monoclonal (a) or polyclonal (b) anti-human ϵ antiserum was inhibited by various concentrations of unlabelled chimaeric IgE (●) or unlabelled myeloma IgE (■).

Methods. Wells of a Dynatec microtitre plate were coated with a solution containing either $1 \mu\text{g ml}^{-1}$ of a monoclonal mouse anti-human ϵ antibody (antibody RB6-2; given by M. D. Cooper) or $3 \mu\text{g ml}^{-1}$ of a polyclonal sheep anti-human ϵ antiserum (Seward Laboratory). After blocking of unreacted sites with bovine serum albumin, a human myeloma IgE (Serotec) that had been radiolabelled with ^{125}I was incubated in the wells in the presence of different concentrations of either chimaeric IgE or unlabelled myeloma IgE itself.

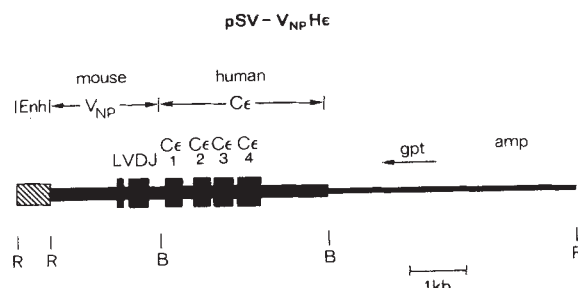


Fig. 1 Structure of plasmid pSV- $V_{NP}H_{\epsilon}$. Immunoglobulin DNA is represented by thick lines, pSV2gpt vector sequences by thin lines and the mouse C_H locus transcription enhancer element by a hatched box. Restriction endonuclease cleavage sites: R, *EcoRI*; B, *BamHI*.

Methods. The *EcoRI*-*BamHI* fragment of plasmid pSV- $V_{\mu 1}$ (ref. 4), which includes the V_H exons, was cloned between the *EcoRI* and *BamHI* sites of plasmid pSV2gpt⁶ to yield pSV- V_{NP} . The *BamHI* fragment of phage $\lambda \epsilon 1.2$ (ref. 14), which includes exons $C_{\epsilon 1}$ to $C_{\epsilon 4}$ of the human ϵ gene, was cloned into pSV- V_{NP} , and plasmid pSV- $V_{NP}H_{\epsilon}$ completed by including in its unique *EcoRI* site the *XbaI*-*EcoRI* enhancer-containing fragment of the mouse heavy-chain locus.

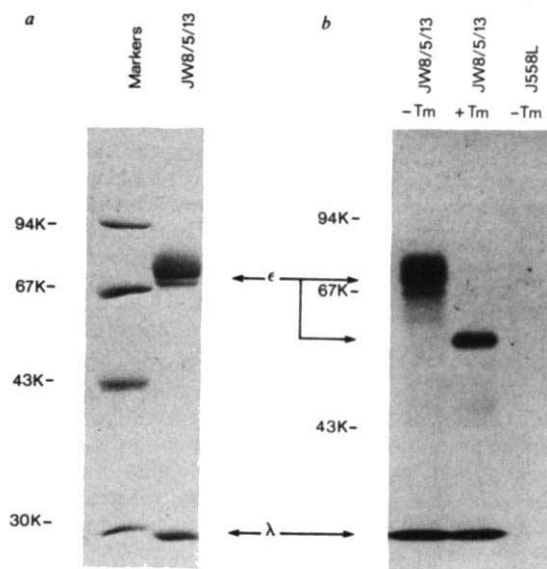


Fig. 3 Analysis of chimaeric IgE by SDS-polyacrylamide gel electrophoresis. a, Purified antibody (30 μg) from a cloned pSV- $V_{NP}H_{\epsilon}$ transfectant of J558L (JW8/5/13) was boiled in the presence of 2-mercaptoethanol, subjected to electrophoresis through a 9% gel and stained with Coomassie blue. b, Biosynthetically labelled antibody from either J558L or the transfectant JW8/5/13 was purified on NIP-Sephrose columns and analysed on a 7% gel after reduction. Samples purified from cells labelled in the presence of tunicamycin are marked +Tm. The positions of relative molecular mass markers are indicated (94K = relative molecular mass 94,000). Biosynthetic labelling with [^{35}S]methionine and purification of both labelled and unlabelled samples on hapten-Sephrose columns were performed as described previously⁹.

Table 1 Histamine release triggered from human basophils passively sensitized with chimaeric IgE

Antigen	% Histamine release ($\pm$ s.e.m.)
—	6.4 $\pm$ 0.8
10 μ g ml ⁻¹ NIP-BSA	18.4 $\pm$ 2.4
1 μ g ml ⁻¹ NIP-BSA	36.0 $\pm$ 5.6
0.1 μ g ml ⁻¹ NIP-BSA	31.2 $\pm$ 12.0
0.01 μ g ml ⁻¹ NIP-BSA	26.4 $\pm$ 2.4
0.001 μ g ml ⁻¹ NIP-BSA	7.2 $\pm$ 1.6
0.0001 μ g ml ⁻¹ NIP-BSA	5.2 $\pm$ 1.2
20 μ g ml ⁻¹ Antigen P1	4.5 $\pm$ 0*

Mononuclear cells were prepared from peripheral blood by sedimentation through dextran-EDTA¹¹; these preparations contained 1–2% basophils. Cells were incubated in duplicate at 37 °C for 2 h in complete Tyrode's buffer¹² with 1.5 μ g ml⁻¹ chimaeric anti-NP IgE. Cells were then centrifuged and resuspended in complete Tyrode's buffer containing the indicated concentrations of (NIP)₃₀-BSA. After 15 min, the histamine released into the supernatant was extracted and assayed fluorimetrically¹¹. The maximum histamine release that could be obtained by incubating the cells at 100 °C was 62.5 ng ml⁻¹. The results are expressed as percentage histamine release: (antigen-induced histamine release [ng ml⁻¹]) $\times$ 100/maximum histamine release obtainable (ng ml⁻¹). The mean background percentage release obtained in repeated experiments was 6.0 $\pm$ 2.5. Both NIP-BSA (1.0 μ g ml⁻¹) and antigen P1 (20 μ g ml⁻¹) alone repeatedly failed to induce histamine release above background levels.

* Data obtained in a separate experiment using cells from the same donor.

IgE itself, probably because the purified chimaeric antibody is essentially homogeneous (see Fig. 3), whereas the commercial sample of myeloma IgE is not.

The structure of the chimaeric protein was investigated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Examination of reduced anti-NP IgE reveals a band co-migrating with mouse λ 1 light chains and a diffuse heavy-chain band of relative molecular mass (M_r) $\sim$ 72,000 (72K) (Fig. 3a); this is considerably larger than the M_r of the ϵ polypeptide chain as predicted from the DNA sequence. However, this discrepancy is resolved by SDS-PAGE of biosynthetically labelled anti-NP IgE secreted by cells labelled in the presence of tunicamycin (Fig. 3b). Incubation with this glycosylation inhibitor reduces the apparent relative molecular mass of the secreted anti-NP IgE from 72K to the predicted value of 62K. Thus the chimaeric IgE, like IgE from human myeloma¹⁰, is heavily glycosylated. The electrophoretic mobility of unreduced anti-NP IgE in SDS-polyacrylamide gels (not shown) is consistent with it having an $\epsilon_2\lambda_2$ structure.

Two types of assay were performed to determine whether the chimaeric anti-NP IgE antibody exhibits the physiological effector functions of authentic human IgE. In one type of assay, we tested the ability of the antibody to trigger histamine release from human basophils. A preparation of mononuclear cells from peripheral blood containing 1–2% basophils was passively sensitized with the chimaeric antibody before incubation with hapten (5-iodo-4-hydroxy-3-nitrophenacetyl caproate, NIP) coupled to bovine serum albumin (NIP-BSA). The results (Table 1) indicate that, after preincubation of basophils with the chimaeric IgE, NIP-BSA is able to trigger a dose-dependent release of histamine. A heterologous antigen, antigen P1 of the house dust mite *Dermatophagoides pteronyssinus*, failed to induce histamine release. Similarly, only background levels of histamine release were obtained with either the chimaeric antibody or NIP-BSA alone. These results indicate that the anti-NP IgE, like authentic human IgE, will not cause degranulation by itself, but will trigger histamine release following crosslinking with antigen.

In a second assay, we demonstrated that incubation with anti-NP IgE could also block the subsequent passive sensitization of basophils by atopic sera containing high IgE levels.

Table 2 Blocking of histamine release by preincubation with chimaeric IgE

Concentration (μ g ml ⁻¹) of chimaeric anti-NP IgE in preincubation	Serum from allergic subject	Antigen P1	% Histamine release ($\pm$ s.e.m.)
—	+	+	20.5 $\pm$ 2.7
0.01	+	+	16.9 $\pm$ 2.4
0.1	+	+	7.2 $\pm$ 0
1	+	+	8.2 $\pm$ 1.0
10	+	+	7.2 $\pm$ 1.0
10	—	—	8.4 $\pm$ 1.2
—	—	+	7.5 $\pm$ 1.7

Mononuclear cells were prepared as described in Table 1. A 2-h preincubation with different concentrations of chimaeric anti-NP IgE was followed by a 2-h incubation with allergic serum (diluted 1:30 in complete Tyrode's), prior to a 15-min exposure to antigen P1 (20 μ g ml⁻¹). The allergic serum used had a total IgE level of 4,800 IU ml⁻¹, and 1,600 BA units ml⁻¹ of IgE specific for *D. pteronyssinus* antigen P1 (ref. 13). The maximum histamine release obtained by incubating the cells used in this experiment at 100 °C was 10.4 ng ml⁻¹.

Table 2 shows that house dust mite antigen P1 can induce the release of histamine from basophils preincubated with serum containing anti-P1 IgE antibody. However, this same serum failed to effect histamine release when the cells had been incubated previously with the chimaeric antibody at concentrations >0.1 μ g ml⁻¹.

The results described here demonstrate that transfection of DNA into mouse myeloma cells is an effective way of producing large amounts of chimaeric antibodies in which mouse V regions provide antigen-binding specificity and human C_H regions provide human effector functions. The known antigen-binding specificity of such an antibody makes its purification extremely simple. Production of a chimaeric IgE in this way has proved particularly attractive as no monoclonal human IgE of known antigen specificity was previously available. The chimaeric antibody may, therefore, prove useful in routine clinical assays. We have demonstrated that this monoclonal IgE is able to block the release of histamine from human basophils which can be triggered *in vitro* by sera from allergic subjects. It will clearly be important to discover whether analogous blocking can be achieved *in vivo* using skin sensitization assays.

We thank S. Morrison for the J558L myeloma, M. D. Cooper for the monoclonal anti- ϵ antibody and R. R. Coombs for critical reading of the manuscript.

Note added in proof: Morrison *et al.*¹⁵ and Boulianne *et al.*¹⁶ have recently described the construction of chimaeric IgG antibodies.

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- Möller, G. (ed.) *Immun. Rev.* **41** (1978).
- Stanworth, D. R., Humphrey, J. H., Bennich, H. & Johansson, S. G. O. *Lancet* **ii**, 330–332 (1967).
- Oi, V. T., Morrison, S. L., Herzenberg, L. A. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **80**, 825–829 (1983).
- Neuberger, M. S. *EMBO J.* **2**, 1373–1378 (1983).
- Ochi, T. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 6351–6355 (1983).
- Mulligan, R. C. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2072–2076 (1983).
- Banerji, J., Olson, L. A. & Schaffner, W. *Cell* **33**, 729–740 (1983).
- Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 717–728 (1983).
- Neuberger, M. S., Williams, G. T. & Fox, R. O. *Nature* **312**, 604–608 (1984).
- Johansson, S. G. O. & Bennich, H. *Immunology* **13**, 381 (1967).
- Lichtenstein, L. M. & Osler, A. G. *J. exp. Med.* **120**, 507–530 (1964).
- Ennis, M. *Ag. Actions* **12**, 60–63 (1982).
- Chapman, M. D. & Platts-Mills, T. A. E. *J. Immun.* **125**, 587–592 (1980).
- Flanagan, J. G. & Rabbitts, T. H. *EMBO J.* **1**, 655–660 (1982).
- Morrison, S. L., Johnson, M. J., Herzenberg, L. A. & Oi, V. T. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6851–6855 (1984).
- Boulianne, G. L., Hozumi, N. & Shulman, M. J. *Nature* **312**, 643–646 (1984).

The gene encoding the T-cell receptor α -chain maps close to the *Np-2* locus on mouse chromosome 14

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Serological and molecular genetic analyses of T-cell clones have shown that the T-cell antigen receptor¹ apparently comprises two glycosylated, disulphide-linked polypeptide chains (α and β), both of which span the cell membrane. Cloning of the genes encoding the two chains from mouse and human DNA has shown that the α - and β -chains are composed of variable (V) and conserved (C) regions in agreement with peptide mapping data²⁻¹¹. Gene segments encoding variable and conserved domains of the β -chain have been identified and undergo rearrangements during T-cell differentiation. The genes encoding the α -chain, so far described at the level of complementary DNA clones, also identify DNA rearrangements. Thus, the genes encoding the T-cell receptor show the same structure and dynamic behaviour as immunoglobulin genes, indicating that the two gene families belong to the same supergene family^{5,12,13}; this evolutionary relationship is supported by the fact that the genes encoding the β -chain of the T-cell receptor are closely linked to immunoglobulin κ light-chain genes on chromosome 6 in mouse¹⁴. In man, however, the β genes map to chromosome 7 (ref. 14) whereas the κ -chain genes are located on chromosome 2, indicating that linkage between the two gene families is not needed for proper expression. Here we describe genomic clones encoding the constant portion of the T-cell receptor α -chain and map the gene to chromosome 14 in mouse, close to the gene for purine nucleoside phosphorylase (*Np-2*) which, in man, has been associated with T-cell immunodeficiencies.

The strategy used to clone the mouse α -chain gene involved the synthesis of a 33-nucleotide long oligomer based on published sequences^{9,10} of α -chain cDNA clones. The oligomer, corresponding to the 5' end of the constant region, was used to screen a BALB/c mouse thymocyte cDNA library constructed in the phage vector λ gt11 and consisting of $\sim 10^6$ independent clones. About 30 positive clones were scored, one of which was picked, rescreened, grown up and subcloned in the plasmid vector pUC9. This clone (T1.2), containing a cDNA insert of $\sim 1,100$ base pairs (bp), was then characterized with five different restriction enzymes; the map obtained (Fig. 1) agrees completely with those published for α -chain cDNA clones^{9,10}. The comparison also indicated that clone T1.2 contains V, joining (J), C and 3'-untranslated region sequences. DNA sequence determination of a portion of the variable and constant region of T1.2 further confirmed its identity as an α -chain cDNA clone (K. Karjalainen, personal communication).

The *Nco*I fragment (probe 1 in Fig. 1) was then used to screen a mouse cosmid library constructed with BDFL 1.1 DNA. BDFL 1.1 is a cytotoxic T-cell clone obtained from a (C57BL/6J $\times$ DBA/2J)F₁ mouse and is specific for fluorescein in association with H-2D^d (W. Haas, unpublished data); 28 positive clones were detected initially, 5 of which were selected finally after rescreening with probe 2 (see Fig. 1). Mapping of these 5 cosmid clones with 14 different restriction enzymes showed that they all contain overlapping DNA sequences. Four enzymes, however, detected four polymorphic restriction sites; these sites defined two groups of overlapping cosmid clones, presumably representing the two constant-region alleles present in BDFL 1.1. Figure 2 shows one clone for each group of cosmid clones representing the two alleles; both of these clones contain the gene for the constant region of the α -chain, as shown by

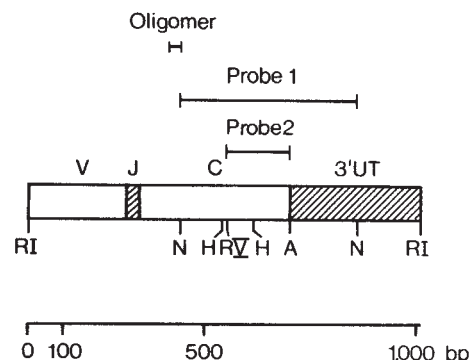


Fig. 1 Restriction enzyme map of the insert of cDNA clone T1.2. Restriction enzymes are: A, *Ava*II; H, *Hind*III; RI, *Eco*RI; RV, *Eco*RV. The *Hind*III site in the pUC9 vector molecule is located to the left. The locations of the oligomer and probes 1 and 2 are indicated.

Methods. The cDNA library was constructed with BALB/c thymocyte mRNA in λ gt11 as described elsewhere²⁸ and screened with a 33-nucleotide long oligomer corresponding to amino-acid residues 141-151 (ref. 9) of the constant region of the T-cell receptor α -chain. The oligonucleotide was synthesized on controlled-pore glass as support, using phosphite chemistry²⁹. Hybridization with T4 kinase-labelled oligomer was done in 50% formamide, 5 $\times$ SSC, 0.1% SDS, 5 $\times$ Denhardt's solution, 10% dextran sulphate, 50 mM sodium phosphate (pH 7), 1 mM sodium pyrophosphate, 100 μ M ATP, 50 μ g ml⁻¹ *Escherichia coli* DNA and 50 μ g ml⁻¹ poly (A) at 42 $^{\circ}$ C overnight. Filters were washed in 6 $\times$ SSC, 0.1% SDS twice for 20 min each at 42 $^{\circ}$ C and exposed to X-ray film. Of 30 positive clones, one was picked, rescreened, grown up and the *Eco*RI insert subcloned into pUC9 using standard procedures³⁰. The clone (T1.2) was mapped by single and double digestions using the restriction enzymes indicated.

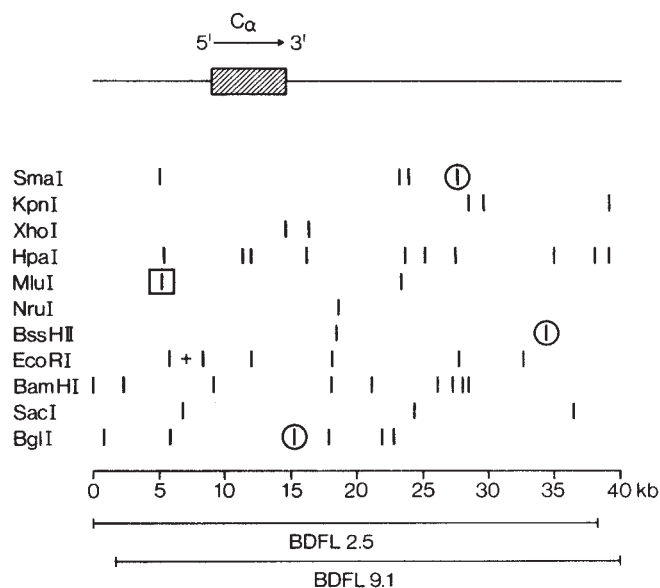


Fig. 2 Molecular map of a 40-kb region of DNA around the constant-region gene of the T-cell receptor α -chain. The region is defined by the two cosmid clones BDFL 2.5 and BDFL 9.1 derived, respectively, from the DBA/2 and the C57BL/6 chromosome of the cytotoxic T-cell clone BDFL 1.1 (see text). Restriction enzymes sites are indicated by vertical bars; those specific for the C57BL/6 allele are circled and the DBA/2-specific site is indicated by a square. No sites were found for *Sal*I, *Cl*aI or *Sac*II. + Indicates that one additional *Eco*RI site is present in the segment marked. The extent of the C_{α} gene is only approximate and was determined by Southern blot hybridization of restriction fragments from the cosmid clones with the probes described in Fig. 1 legend. The cosmid library of BDFL 1.1 DNA was constructed using the vector pNNL and screened with oligo-labelled³¹ probe 1 (Fig. 1) as described elsewhere³². Cosmid clones were analysed by restriction enzyme digestion and hybridization as described^{32,33}.

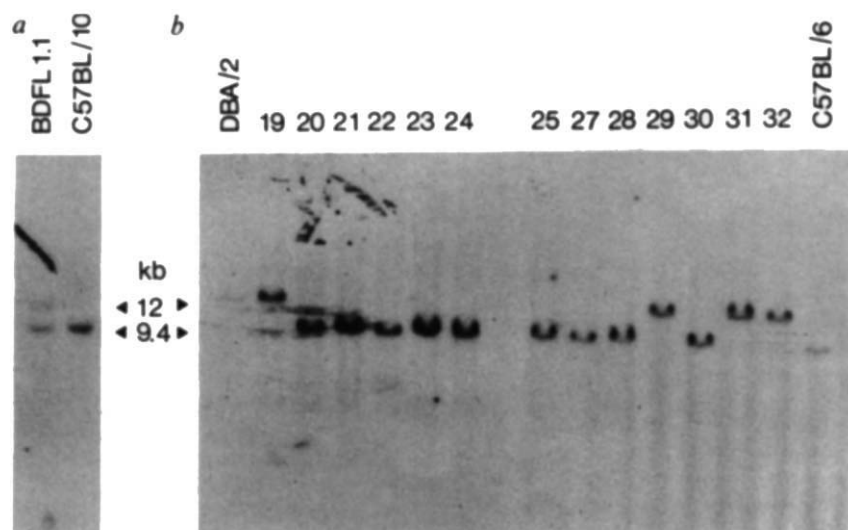


Fig. 3 Southern blot analysis of *Bgl*I-digested mouse DNA with a C_{α} probe. *a*, DNA from C57BL/10 mouse liver and the BDFL 1.1 cell clone. *b*, DNA from DBA/2 and C57BL/6 mouse livers and 13 recombinant inbred strains of the BXD series (see Table 1). DNA (5–10 μ g of each sample) was digested to completion and separated on a 0.6% agarose gel, transferred to nitrocellulose filters and hybridized with oligo-labelled³¹ probe 2 (Fig. 1) as described previously³³. BXD DNAs were obtained from the Jackson Laboratory.

hybridization with the cDNA probes 1 and 2 and the synthetic oligomer. Hybridization of distinct restriction fragments to the oligomer (5' probe) and the *Nco*I fragment (3' probe) defined the 5' to 3' orientation of the constant-region gene (Fig. 2). As DNA rearrangements, resulting from *V-J* joining, are presumed to occur upstream of the constant-region gene, we are uncertain as to whether the polymorphism revealed by the *Mlu*I site is a result of a possible *V-J* joining on one or both chromosomes.

To confirm that the polymorphic restriction sites on cosmid clones 9.1 and 2.5 do indeed characterize the α -chain alleles derived from DBA/2 and C57BL/6 DNA, we performed Southern blotting experiments using probe 2. As expected from the cosmid clones, two *Bgl*I fragments of 12 and 9.4 kilobases (kb) were identified in BDFL 1.1 DNA (Fig. 3*a*). These fragments were identical in size to the 12-kb *Bgl*I fragment detected in DBA/2 DNA and the 9.4-kb fragment found in C57BL/10 or C57BL/6 (Fig. 3*b*) DNA, indicating that cosmid clone 2.5 corresponds to the DBA/2 allele whereas cosmid clone 9.1 represents the C57BL/6 allele.

The restriction site polymorphism revealed between DBA/2 and C57BL/6 DNA immediately suggested the use of recombinant inbred strains of mice to determine the location of the α -chain gene in the genome of the mouse. Recombinant inbred

strains are derived by crossing two genetically distinct progenitor strains and establishing multiple independent strains from the F_2 generation by continuous brother-sister inbreeding¹⁵. The chromosomes of recombinant inbred strains consist of alternating segments derived from the two progenitor strains, the boundaries of which are determined by chance fixation of crossovers during the inbreeding process. Once inbred, the recombinant strains are typed for genetic loci that discriminate between the progenitors to obtain a strain distribution pattern characteristic for each locus. As closely linked genes will tend to become fixed in the same combination as they entered the cross, recombinant inbred strains are useful for detecting linkages and for estimating recombination frequencies.

The BXD series of recombinant inbred strains consists of 26 strains derived from crossing C57BL/6J with DBA/2J mice¹⁶. Over 150 loci have now been typed. We tested DNA of the 26 strains after *Bgl*I digestion with probe 2 and obtained the strain distribution pattern for the α -chain gene shown in Fig. 3*b* and Table 1. This pattern shows perfect concordance with the strain distribution pattern of the *Np-2* locus, indicating with 95% certainty that the α -chain gene maps within 3.3 centimorgans of *Np-2* (ref. 17). The probability of finding complete concordance for two unlinked loci by chance alone is $<1 \times 10^{-7}$ in the BXD series.

The *Np-2* locus is closely linked to the *Np-1* locus¹⁸ on chromosome 14 in the mouse¹⁹. Thus, neither the α - nor the β -chain gene for the T-cell receptor is located on chromosome 12 in the mouse, which carries the immunoglobulin heavy-chain genes. The finding of immunoglobulin allotype-linked idiotypes on T cells therefore remains unexplained^{20,21}.

In C57BL mice, *Np-2* controls an erythrocyte-specific isozyme of purine nucleoside phosphorylase, an enzyme important for the catabolism of purine nucleotides²². The common form of this enzyme found in all inbred strains studied, including C57BL, is encoded by the *Np-1* locus. Interestingly, in man, deficiencies of purine nucleoside phosphorylase have been shown to be associated with severe T-cell immunodeficiencies²³. It has been proposed that elevated levels of dGTP accumulating in the absence of the enzyme are toxic to T lymphocytes²⁴. Given the apparent close linkage between the genes for the T-cell receptor α -chain and nucleoside phosphorylase in the mouse, it is likely that the human α -chain gene is also linked to the nucleoside phosphorylase locus²⁵. If this is the case, at least some of the phosphorylase-linked T-cell deficiencies in man might be the result of genetic defects affecting both loci; this can be tested as the location of the phosphorylase gene in man is known (incidentally also on chromosome 14; ref. 26) and the gene has been cloned²⁷.

Complete concordance between the strain distribution patterns for the *Np-2* locus and the α -chain gene is also observed

Table 1 Strain distribution pattern of the *Bgl*I polymorphism of the α gene

Strain	Restriction fragment	Strain	Restriction fragment
BDFL 1.1	12 kb(D), 9.4 kb(B)	BXD 16	D
DBA/2	D	BXD 18	D
C57BL/10	B	BXD 19	D
C57BL/6	B	BXD 20	B
BXD 1	B	BXD 21	B
BXD 2	D	BXD 22	B
BXD 5	B	BXD 23	B
BXD 6	D	BXD 24	B
BXD 8	D	BXD 25	B
BXD 9	D	BXD 27	B
BXD 11	D	BXD 28	B
BXD 12	D	BXD 29	D
BXD 13	B	BXD 30	B
BXD 14	D	BXD 31	D
BXD 15	D	BXD 32	D

*Bgl*I fragments detected with probe 2 (Fig. 1) in the various recombinant inbred strains listed were either 12 kb (derived from DBA/2, D allele) or 9.4 kb (derived from C57BL/6, B allele) long. The heterozygous BDFL 1.1 cell clone (C57BL/6 $\times$ DBA/2) contained a 12- and a 9.4-kb *Bgl*I fragment (compare with Fig. 3).

in the BXH (12 strains) and CXB (7 strains) series of recombinant inbred strains. Furthermore, the two *H*-8 congenic mouse strains, B6.C-H-8^c/By and B10.D2 (57N)/Sn, contain the BALB/c and DBA/2 alleles, respectively, at both the *Np*-2 and α -chain gene loci. We propose *Tcra* as a symbol for the T-cell receptor α -chain gene locus.

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containing *Bgl*I-digested BXD DNAs, K. Fischer Lindahl and H. v. Boehmer for critically reading the manuscript, K. Karjalainen for discussions, H. P. Stahlberger for artwork and Lynn Gisler for preparation of the manuscript. Part of the work was supported by NIH grant GM 18684 to B.A.T. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care. The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche, Ltd.

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- Robertson, M. *Nature* **312**, 16-17 (1984).
- Acuto, O. *et al. Cell* **34**, 717-726 (1983).
- Kappler, J. *et al. Cell* **35**, 295-302 (1983).
- Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. *Nature* **308**, 149-153 (1984).
- Yanagi, Y. *et al. Nature* **308**, 145-149 (1984).
- Siu, G. *et al. Cell* **37**, 393-401 (1984).
- Malissen, M. *et al. Cell* **37**, 1101-1110 (1984).
- Gascoigne, N. R. J., Chien, Y., Becker, D. M., Kavalier, J. & Davis, M. M. *Nature* **310**, 387-391 (1984).
- Chien, Y. *et al. Nature* **312**, 31-35 (1984).
- Saito, H. *et al. Nature* **312**, 36-40 (1984).
- Hannum, C. H., Kappler, J. W., Trowbridge, I. S., Marrack, P. & Freed, J. *Nature* **312**, 65-67 (1984).
- Hedrick, S. M., Nielsen, E. A., Kavalier, J., Cohen, D. I. & Davis, M. M. *Nature* **308**, 153-158 (1984).
- Siu, G. *et al. Nature* **311**, 344-350 (1984).
- Caccia, N. *et al. Cell* **37**, 1090-1099 (1984).
- Bailey, D. W. in *The Mouse in Biomedical Research* Vol. 1 (eds Foster, H. L., Small, J. D. & Fox, J. G.) 223-239 (Academic, New York, 1981).
- Taylor, B. A. in *Genetic Variants and Strains of the Laboratory Mouse* (ed. Green, M. C.) 397-407 (Fischer, Stuttgart, 1979).

- Taylor, B. A. in *Origins of Inbred Mice* (ed. Morse, H. C. III) 423-428 (Academic, New York, 1978).
- Taylor, B. A. *Mouse News Lett.* **65**, 28 (1981).
- Womack, J. E., Davisson, M. T., Eicher, E. M. & Kendall, D. A. *Biochem. Genet.* **15**, 347-355 (1977).
- Binz, H., Wigzell, H. & Bazin, H. *Nature* **264**, 639-642 (1976).
- Rajewski, K. & Eichmann, K. *Contemp. Topics Immunobiol.* **7**, 69-112 (1977).
- Bremner, T. A., Premkumar-Reddy, E., Naylor, K. & Kouri, R. E. *Biochem. Genet.* **16**, 1143-1151 (1978).
- Giblett, E. L., Ammann, A. J., Wara, D. W., Sandman, R. & Diamond, L. K. *Lancet* **i**, 1010-1013 (1975).
- Gudas, L. J., Ullman, B., Cohen, A. & Martin, D. W. Jr *Cell* **14**, 531-538 (1978).
- Nadeau, J. H. & Taylor, B. A. *Proc. natn. Acad. Sci. U.S.A.* **81**, 814-818 (1984).
- George, D. L. & Francke, U. *Science* **194**, 851-852 (1976).
- Williams, S. R., Goddard, J. M. & Martin, D. W. Jr *Nucleic Acids Res.* **12**, 5779-5787 (1984).
- Goridis, C. *et al. EMBO J.* (in the press).
- Kiefer, H. R. & Bannwarth, W. in *Immunological Methods* Vol. 3 (eds Lefkovits, I. & Pernis, B.) (Academic, New York, in the press).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1983).
- Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **137**, 266-267 (1984).
- Steinmetz, M., Stephan, D., Dastoor-nikoo, G. R., Gibb, E. & Romaniuk, R. in *Immunological Methods* Vol. 3 (eds Lefkovits, I. & Pernis, B.) (Academic, New York, in the press).
- Steinmetz, M. *et al. Nature* **300**, 35-42 (1982).

The human T-cell receptor α -chain gene maps to chromosome 14

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The T-cell receptor for antigen has been identified as a disulphide-linked heterodimeric glycoprotein of relative molecular mass (*M*_r) 90,000 comprising an α - and a β -chain¹⁻³. The availability of complementary DNA clones encoding mouse⁴ and human⁵ β -chains has allowed a detailed characterization of the genomic organization of the β -chain gene family and has revealed that functional β -chain genes in T cells are generated from recombination events involving variable (*V*), diversity (*D*), joining (*J*) and constant (*C*) gene segments^{6,7}. Recently, cDNA clones encoding mouse^{8,9} and human¹⁰ α -chains have been described; the sequences of these clones have indicated that functional α -chain genes are also generated from multiple gene segments. It is possible that chromosomal translocations involving T-cell receptor α - and β -chain genes have a role in T-cell neoplasms in much the same way as translocations involving immunoglobulin genes are associated with oncogenic transformation in B cells¹¹. In the latter case, the chromosomal localization of the immunoglobulin genes provided one of the first indications of the involvement of such translocations in oncogenic transformation. The chromosomal assignment of the α - and β -chain genes may, therefore, provide equally important clues for T-cell neoplastic transformation. The chromosomal location of the mouse and human β -chain gene family has been determined: the murine gene lies on chromosome 6 (refs 12, 13) whereas the human gene is located on chromosome 7 (refs 13, 14). Here we use a cDNA clone encoding the human α -chain to map the corresponding gene to chromosome 14.

The α -chain cDNA clone used in this study was isolated from a ϕ gt10 library, constructed from messenger RNA isolated from the human T-cell line Jurkat¹⁵, by cross-hybridization with a homologous mouse probe⁸. The sequence of the insert corresponds exactly to that of a previously published α -chain cDNA clone isolated from HPB-MLT¹⁰ and comprises about three-quarters of the constant region plus almost the entire 3'-untranslated region of the human α -chain (M.K.L.C., M.J.O., G.T. and S.T., manuscript in preparation).

The insert (~900 base pairs, bp) from the ϕ gt10 clone was subcloned into pBR322 and the resulting plasmid (pJ6 α 2) used to screen human and mouse DNA, and a panel of mouse/human somatic cell hybrid DNAs using Southern hybridization analysis. Human DNA digested with *Hind*III and hybridized with the pJ6 α 2 insert shows two bands of 4.2 and 2.9 kilobases (kb)

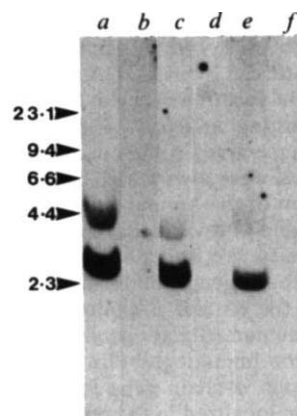


Fig. 1 Southern hybridization analysis of mouse/human somatic cell hybrid DNA. DNA of high *M*_r was prepared according to Maniatis *et al.*³⁴ and 20 μ g was digested with *Hind*III and electrophoresed through 0.8% agarose, blotted onto nitrocellulose and hybridized with oligo-labelled³⁵ insert from pJ6 α 2. Hybridization was carried out at 65 °C in 3 $\times$ SSC. The filters were washed at 65 °C in 0.2 $\times$ SSC. The sizes of the DNA fragments were determined by reference to the mobilities of ϕ lHindIII markers. *a*, Human fibroblast (Bu) DNA; *b*, mouse L-cell (1R) DNA; *c*, hybrid F4SC13 C19 DNA; *d*, hybrid HORL411B6P DNA; *e*, hybrid FIR5R3 DNA; *f*, hybrid DT1.2R DNA.

Table 1 Segregation of a human α -chain gene in somatic cell hybrids

Hybrid (ref.)	Human chromosomal constitution	Presence of human-specific bands*
DUR4.3 (25)	3, 10, 11, 12, 13, 14, 15, 17, 18, 20, 21, 22, X	+
CTP34B4 (26)	1, 2, 3, 5, 6, 7, 8, 12, 14, 16, 17, 18, X	+
SIR74ii (27)	1, 2, 4, 12, 14, 17, 18, 20, 21, 22, X	+
3W4 C15 (28)	7, 10, 11, 12, 14, 15, 17, 21, X	+
HORP27R C14 (29)	4, 7, 11, 12, 14, 15, 21, X	+
CTP41-2 (30)	2, 7, 8, 14, 16, 17, X	+
F4SC13 C19 (31)	1, 14, X	+
FIR5R3 (32)	14, 18	+
DT1.2R (25)	3, 10, 11, 17, 18, 20, 21, 22	-
HORL411B6P (29)	1, 3, 11, 18, 22, X	-
CI 21 (33)	7	-
Controls		
1R (28)	Mouse L-cell	-
Bu†	Human fibroblast	+

Note that several of the hybrids have been recloned and recharacterized since their description in the original reference. Human chromosomal constitution was determined by a combination of karyotypic²⁵, isozyme³⁰ and antigenic analysis³⁰. Batches of cells for DNA production were checked by isozyme analysis.

* The hybridization conditions are given in Fig. 1 legend. Under conditions of high stringency only the 4.2- and 2.9-kb human bands were detected. In all cases where the hybrids were scored positive both bands were visible.

† A human fibroblast line derived from a human ovarian teratoma. This line was obtained from Dr S. Povey (MRC Biochemical Genetics Unit, London).

(Fig. 1a). This result is consistent with the nucleotide sequence of the human α -chain (ref. 10 and G.T. and S.T., unpublished observation), in which a *Hind*III site is located in the constant region, and suggests that a single constant-region gene segment is present in the human genome. One hybridizing band of 6 kb was detected with *Hind*III-digested mouse DNA probed at low stringency (data not shown). As the 4.2- and 2.9-kb bands were apparently specific to human DNA and the mouse-specific bands could be removed by washing at high stringency (Fig. 1b), the presence of the 4.2-kb and 2.9-kb fragments in *Hind*III-digested mouse/human somatic cell hybrid DNA could be used to determine the chromosomal location of the α -chain gene. Figure 1c-f shows Southern blotting analysis of DNA from four such hybrids. For hybrids positive for the human α -chain gene, both human-specific bands were always detected (see, for example, Fig. 1c, e). Inspection of the human chromosomal content of a panel of hybrids (Table 1) reveals that the human α -chain gene maps to chromosome 14. We, and others, have shown previously that the human β -chain gene family maps to chromosome 7 (refs 13, 14). Thus, the α - and β -chain genes lie on different chromosomes. The human β -chain genes also lie on a different chromosome from the immunoglobulin *Igh*, *Igk* and *Igl* loci. In contrast, the human α -chain gene is syntenic with the *Igh* loci, which have been localized in the region 14q32.33-14qter¹⁶. The human oncogene *c-fos* has also been localized to chromosome 14 in the region 14q21-q31¹⁷.

A conserved linkage group between human chromosome 14 and mouse chromosome 14 occurs at the nucleoside phosphorylase (*Np*) locus (at 14q13.1 in human; reviewed in ref. 18). In man, nucleoside phosphorylase deficiency has been associated with severe T-cell immunodeficiency¹⁹. Interestingly, the gene encoding the mouse α -chain has been mapped to chromosome 14, close to the *Np-2* locus^{20,21}. This observation suggests that the human α -chain gene may also lie close to the *Np* locus in a conserved linkage group. If this is the case, the human α -chain

gene, although on the same chromosome, must reside a considerable distance from the *Igh* locus.

Abnormalities of chromosome 14 have been associated with human B-lymphocyte malignancies. Thus, Burkitt lymphomas are often associated with 8;14 translocations resulting in a juxtaposition of the *c-myc* and *Igh* loci¹¹. Inversions of chromosome 14 due to breaks near q11 and q32 are found in T-cell chronic lymphocytic leukaemia and lymphomas, and simple translocations involving chromosome 14 have also been observed in T-cell malignancies²²⁻²⁴. For example, a translocation involving chromosomes 10 and 14 with a breakpoint at 14q11.2 has been found in a T-cell lymphoma cell line²⁴. The involvement of the 14q11 region in these inversions and translocations suggests that it is related to T-cell function and that rearrangements involving this region may be involved in T-cell malignancies. If the human α -chain gene is located, by analogy with the linkage in the mouse, at or near the *Np* locus, it would be a candidate for such a gene. We are presently performing *in situ* hybridization analysis to test this possibility.

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- Allison, J. P., McIntyre, B. W. & Bloch, D. J. *Immun.* **129**, 2293-2300 (1982).
- Haskins, K. et al. *J. exp. Med.* **157**, 1149-1169 (1983).
- Meuer, S. C. et al. *J. exp. Med.* **157**, 705-719 (1983).
- Hedrick, S. M., Nielsen, E. A., Kavalier, J., Cohen, D. I. & Davis, M. M. *Nature* **308**, 153-158 (1984).
- Yanagi, Y. et al. *Nature* **308**, 145-149 (1984).
- Chien, Y., Gascoigne, N. R. J., Kavalier, J., Lee, N. E. & Davis, M. M. *Nature* **309**, 322-326 (1984).
- Siu, G. et al. *Cell* **37**, 393-401 (1984).
- Saito, H. et al. *Nature* **312**, 36-40 (1984).
- Chien, Y. et al. *Nature* **312**, 31-35 (1984).
- Sim, G. K. et al. *Nature* **312**, 771-775 (1984).
- Perry, R. P. *Cell* **33**, 647-650 (1983).
- Lee, N. E. et al. *J. exp. Med.* **160**, 905-913 (1984).
- Caccia, N. et al. *Cell* **37**, 1091-1099 (1984).
- Collins, M. K. L. et al. *EMBO J.* **3**, 2347-2349 (1984).
- Collins, M. K. L. et al. *EMBO J.* (submitted).
- Croce, C. M. et al. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3416-3419 (1979).
- Barker, P. E. et al. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5826-5830 (1984).
- Ferguson-Smith, M. A. & Cox, D. R. *Cytogenet. Cell Genet.* **37**, 127-154 (1984).
- Giblett, E. L., Ammann, A. J., Wara, D. W., Sandman, R. & Diamond, L. K. *Lancet* **i**, 1010-1013 (1975).
- Dembic, Z., Bannwarth, W., Taylor, B. A. & Steinmetz, M. *Nature* **314**, 271-273 (1985).
- Kranz, D. et al. *Science* (in the press).
- Zech, L. et al. *Nature* **308**, 858-860 (1984).
- Ueshima, Y., Rowley, J. D., Varikujis, D., Winter, J. & Gordon, L. *Blood* **63**, 1028-1038 (1984).
- Hecht, E., Morgan, R., Hecht, B. K.-M. & Smith, S. D. *Science* **226**, 1445-1447 (1984).
- Solomon, E. et al. *Somat. Cell Genet.* **2**, 125-140 (1976).
- Jones, E. A., Goodfellow, P. N., Kennett, R. H. & Bodmer, W. F. *Somat. Cell Genet.* **2**, 483-496 (1976).
- Whitehead, A. S. et al. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5021-5025 (1982).
- Nabholz, M., Giggiano, J. & Bodmer, W. F. *Nature* **223**, 358-363 (1969).
- Van Heyningen, V. et al. *Ann. hum. Genet.* **38**, 295-303 (1975).
- Tunncliffe, A. & Goodfellow, P. in *Genetic Analysis of the Cell Surface, Receptors and Recognition* Vol. 16 (ed. Goodfellow, P.) 57-82 (Chapman & Hall, London, 1984).
- Sykes, B. & Solomon, E. *Nature* **272**, 548-549 (1978).
- Solomon, E. et al. *Cytogenet. Cell Genet.* **35**, 64-66 (1983).
- Croce, C. M. & Koprowski, H. *J. exp. Med.* **140**, 1221-1229 (1974).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
- Feinberg, A. O. & Vogelstein, B. *Analyt. Biochem.* **137**, 266-267 (1984).
- Harris, H. & Hopkinson, D. A. *Handbook of Enzyme Electrophoresis in Human Genetics* Suppl. 1977-78 (North-Holland, Amsterdam, 1976).

Stimulation of the Na/H exchanger of sea urchin eggs by phorbol ester

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On fertilization of a sea urchin egg, marked changes occur in the cytoplasmic concentration of calcium and hydrogen ions. These ionic signals represent the necessary and sufficient stimuli for the increased metabolism, protein synthesis and DNA synthesis that constitute egg activation¹⁻³. Cytoplasmic alkalization, the major immediate cause of the increased rate of protein synthesis which

occurs at fertilization⁴, arises because the sperm-induced intracellular calcium transient activates a coupled flux of sodium ions and hydrogen ions across the oolemma⁵⁻⁷. The experiments reported here suggest that the second messenger which links the activation of the Na/H exchange to the calcium transient may be a substance which stimulates protein kinase C⁸, as 12-*O*-tetradecanoyl phorbol acetate (TPA), a known activator of protein kinase C⁹, appears to stimulate protein synthesis by turning on the Na/H exchanger and causing a cytoplasmic alkalization. Our data indicate that one consequence of treating other tissues with TPA, a tumour promoter, may be an increase in intracellular pH¹⁰⁻¹².

The synthesis of proteins from maternal mRNA is necessary for the normal development of the sea urchin embryo¹³. Figure 1 shows that a concentration of TPA that supports proliferation in cultured mammalian cells¹⁴ increased the rate of incorporation of amino acid into protein in unfertilized sea urchin eggs (*Lytechinus pictus*). The increased incorporation of amino acid into protein was slower in onset and of lesser magnitude than that stimulated at fertilization or by treatment with the calcium ionophore A23187 (refs 1, 4). Fertilization or treatment with ionophore stimulated a 10-fold increase in the rate of protein synthesis which is first detected 15 min after fertilization, whereas 320 nM TPA caused a fourfold increase in the rate after a lag of 30 min. At fertilization and on treatment with ionophore, calcium is released from a store within the egg and the cytoplasmic calcium concentration increases^{15,16}. In contrast, treatment of eggs with up to 3 μ M TPA caused none of the morphological changes associated with the increased cytoplasmic calcium concentration and consequent cortical granule exocytosis. The cortical granule exocytosis is a sensitive *in situ* assay for an increased intracellular calcium concentration^{15,17}. As no cortical granule exocytosis occurred on treatment with TPA and since TPA itself does not, we have found, inhibit exocytosis *in vitro* or *in vivo* at these concentrations, we infer that no substantial increase in the calcium concentration, such as that which normally occurs at fertilization, occurred when the egg was treated with TPA.

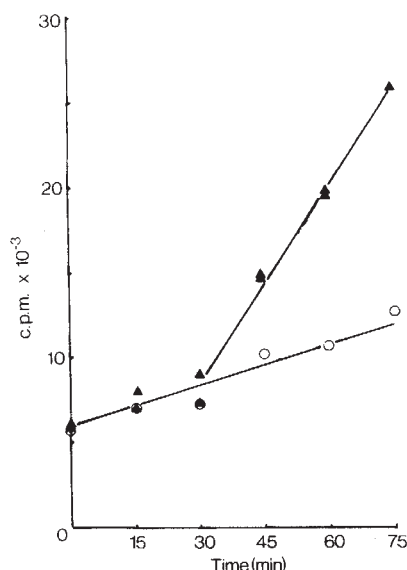


Fig. 1 TPA stimulates the incorporation of [4, 5-³H]leucine into egg trichloroacetic acid (TCA) precipitates. Incorporation was measured using published methods²³. Eggs were pre-loaded with radioactive amino acid and the TCA precipitate collected on glass-fibre filters. ▲, 320 nM TPA (Sigma) was added at $t=0$ to eggs in artificial sea water (450 mM NaCl, 50 mM MgCl₂, 10 mM KCl, 11 mM CaCl₂, 2.5 mM NaHCO₃, pH 8.0). ○, Controls. The data shown are from two experiments. The mean increase in rate of [4, 5-³H]leucine incorporation was 3.2 ± 0.1 -fold (mean $\pm$ s.e.m., $n=4$). TPA-treated eggs have an intact intracellular calcium store as they will raise normal fertilization membranes on insemination or addition of calcium ionophore. Temperature 16 °C.

Other parthenogenetic activators exist which stimulate protein synthesis in sea urchin eggs without releasing the egg's internal calcium store^{4,18}. These are penetrating weak bases which stimulate protein synthesis by raising the egg's cytoplasmic pH⁹. Figure 2 presents data which indicate that TPA may be acting in a similar manner: unfertilized eggs loaded with fluorescein by incubation with fluorescein diacetate show a stable

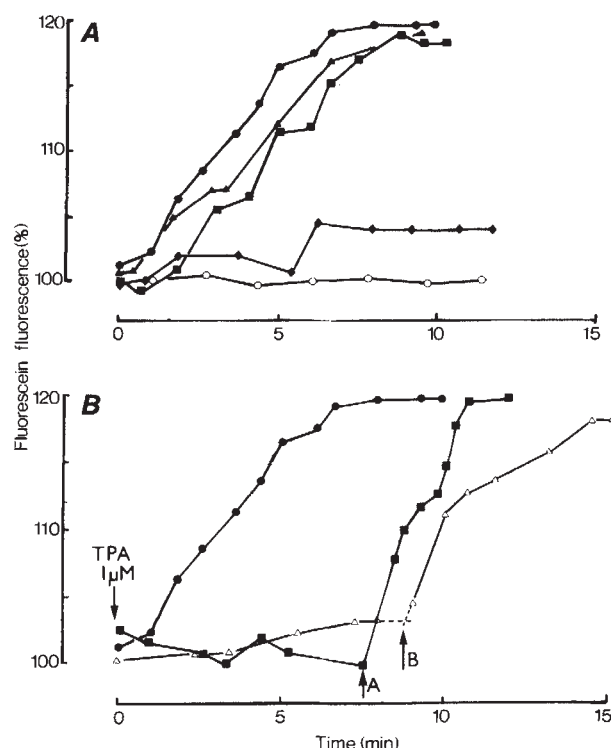


Fig. 2 The increase in fluorescein fluorescence in eggs treated with TPA and its less potent methyl analogue, 4-*O*-methyl-tetradecanoyl phorbol ester. Fluorescein fluorescence was measured in individual eggs using a Leitz Diavert fluorescence microscope and a photomultiplier as described previously². **A**, Extent of the increase in fluorescein fluorescence at various concentrations of TPA: ●, 1,000; ▲, 200; ■, 100; ◆, 50 nM. The increase in fluorescence at maximal TPA concentrations was $117 \pm 1.8\%$ (mean $\pm$ s.e.m., $n=9$). The increase in fluorescein fluorescence at fertilization in identical experimental conditions is $119 \pm 3.1\%$ (mean $\pm$ s.e.m., $n=7$)³. To illustrate the pharmacological specificity of the response, open circles show the lack of response to the less potent analogue, MeTPA. **B**, Inhibition of the response to 1,000 nM TPA (●) by complete replacement of sodium in sea water by choline (■, NaHCO₃ was replaced by KHCO₃) or by addition of 100 μ M dimethylamiloride (Δ). When sodium-containing sea water was re-admitted at the time indicated by the arrow labelled A, or dimethylamiloride removed at the time indicated by arrow B, the response was immediate.

Methods. Eggs were loaded with fluorescein by incubating them in sea water containing 50 μ M fluorescein diacetate (Molecular Probes, Junction City, Oregon) for 3–5 min and washed three times before being lightly attached with polylysine to a coverslip on the microscope stage. Excitation was provided by a mercury vapour lamp provided with a 3 OD neutral density filter and 450–490-nm bandpass filter. Epifluorescent illumination was achieved with a 510-nm dichroic mirror. Emitted light was collected by a photomultiplier in the image plane provided with a 510-nm long-pass filter. The intensity of the 520-nm fluorescein fluorescence peak is sensitive to pH over the physiological range and provides information as to relative changes in pH. Exciting light was applied intermittently to minimize fluorescence bleaching. In these conditions the fluorescence signal remains constant for extended periods (>1 h), indicating that the dye is neither lost from the eggs nor significantly bleached. We suppose that the fluorescence which we measure primarily reflects cytoplasmic pH, an assumption borne out by the similarities between the results obtained with this method and with that using intracellular pH microelectrodes². Temperature 16 °C.

fluorescence signal² which increases when the eggs are treated with 100–1,000 nM TPA. The increase in fluorescein fluorescence is similar in magnitude to that seen at fertilization or after treatment with A23187, and can be attributed to an increase in cytoplasmic pH². Though it might be argued that a proportion of the TPA-induced increase in fluorescein fluorescence is the result of fluorescein uptake into mitochondria²⁰, the absolute requirement for external sodium ions and the identical time course of the fluorescein increase and pH microelectrode potential under a variety of conditions² makes a substantial mitochondrial contribution to the fluorescence increase unlikely. That the TPA-induced increase in intracellular pH requires external sodium ions also suggests, by analogy with fertilization, that it is the exchange of external sodium ions for internal hydrogen ions that is responsible for the cytoplasmic alkalization of TPA-treated eggs. This conclusion was confirmed by the finding that dimethylamiloride, a drug which specifically inhibits electroneutral Na/H exchange²¹, inhibits the TPA-induced cytoplasmic alkalization in eggs (see Fig. 3B).

The activation of the Na/H exchanger at fertilization leads to a transient net efflux of hydrogen ions from the egg^{5–7}. Figure 3 shows that TPA induced an efflux of hydrogen ions that behaved very similarly to the hydrogen ion efflux which occurs at fertilization. Figure 3 also shows that 4-*O*-methyltetradecanoyl phorbol ester (MeTPA), a less potent TPA analogue, was ineffective at these concentrations. The TPA-induced efflux rate was maximal at 2.5 min and had fallen to control levels by 5 min after treatment. The efflux was sodium-dependent; it was sensitive to external sodium ion concentration in the range 0.1–5 mM at pH 8.0. This dependence on external sodium concentration is identical to that of the fertilization-induced Na/H exchange, which also requires only a small fraction of the normal sodium concentration of sea water for full activation²². The TPA-induced hydrogen ion efflux was half-maximal at 100 nM TPA, representing a net efflux of 20 nmol ml⁻¹ of a 1% egg suspension at pH 8.0. The fertilization-induced efflux under similar conditions was 30 nmol ml⁻¹ of a 1% egg suspension. The Na/H exchanger has been reported to contribute three-quarters of the total acid efflux occurring at fertilization⁵ and is inhibited by amiloride^{5,7}. We find that the TPA-induced hydrogen ion efflux is half-maximally inhibited by 40 µM dimethylamiloride, the concentration which half-maximally inhibits A23187-induced hydrogen ion efflux (see Fig. 3B). We conclude that TPA stimulates the Na/H exchanger of unfertilized eggs to extrude a quantity of hydrogen ions equivalent to the quantity extruded at fertilization. Activation of the Na/H exchange is a sufficient explanation of the effects of TPA on the intracellular pH.

A simple hypothesis for the mechanism by which TPA stimulates protein synthesis is that it acts primarily on the Na/H exchanger, and that it is the ensuing increase in intracellular pH which is responsible for the increase in the rate of incorporation of amino acid into protein. This simple hypothesis is supported by the data of Fig. 4. The increase in the rate of incorporation of amino acid into protein induced by TPA is similar to that induced by treatment with the weak base

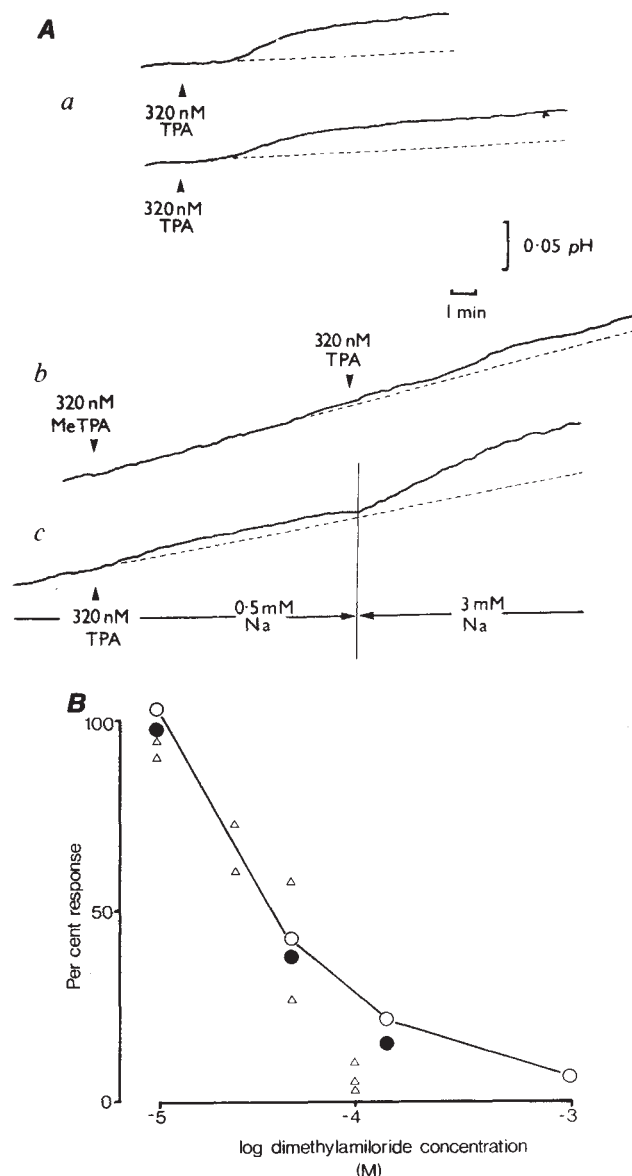


Fig. 3 The hydrogen ion efflux from unfertilized eggs measured with an external pH electrode in a 2% egg suspension in artificial sea water. The sea water was buffered normally and the external pH change kept to a minimum as the extent of the hydrogen ion efflux depends on the external pH⁶. **A:** *a*, Two representative examples of the external acidification observed when the egg suspension was treated with 320 nM TPA (added from a 320 µM stock solution in dimethyl sulphoxide (DMSO); DMSO did not itself induce any of the changes shown in this or the other figures). The extent of the acid efflux was determined by back-titration with sodium hydroxide⁵. *b*, MeTPA (1,000 nM) caused no significant increase in acid efflux. A significant though attenuated response to 320 nM TPA was seen subsequently. *c*, The response to 320 nM TPA in artificial sea water containing 0.5 mM Na (choline-substituted) is one-fifth of the response in normal sea water. Further reduction in the sodium concentration completely abolishes the response (not shown). When sodium was added to increase the sodium concentration to 3 mM externally, the full TPA-induced hydrogen ion efflux was observed. The broken line is an extrapolation of the increase in extracellular pH which occurs in stirred, dense unfertilized egg suspensions in these conditions. Suspensions rapidly attained this linear rate of acid production, which was recorded for 5 min in each experiment before addition of the drug. **B**, The TPA-induced hydrogen ion efflux is inhibited by dimethylamiloride²¹ (●), as is the hydrogen ion efflux caused by the calcium ionophore A23187 (○). Also shown (△) is the inhibition by dimethylamiloride of the TPA-induced fluorescein fluorescence, measured as described in Fig. 2 legend. Sea water containing 50 mM sodium. Dimethylamiloride was given by Dr E. J. Cragoe Jr. Temperature 18 °C.

Table 1 Effect of dimethylamiloride and removal of Na ions on TPA-induced increase in protein synthesis

	c.p.m. in protein at:	
	45 min	60 min
Control	2,110 ± 531	3,690 ± 284
320 nM TPA	4,783 ± 515*	5,265 ± 461†
320 nM TPA + 100 µM dimethylamiloride	2,773 ± 461 (n = 3)*	—
320 nM TPA in Na-free sea water	—	3,592 ± 702†

The c.p.m. values are the mean ± s.e.m. of four experiments of the type described in Fig. 1 legend.

* $P < 0.05$; † $P < 0.01$, Student's *t*-test.

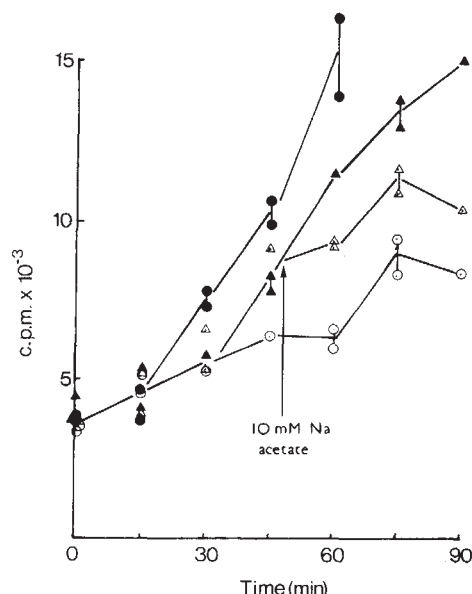


Fig. 4 TPA-induced protein synthesis and intracellular pH. The data are from two experiments. ●, 10 mM NH₄Cl induces a similar increase in the rate of protein synthesis to that obtained on treatment with 320 nM TPA (▲). When 10 mM sodium acetate in artificial sea water (pH 6.5)¹⁹ is added to eggs which have been treated with 320 nM TPA (△), the rate of protein synthesis falls to that of controls (○). Conditions and methods are identical to those of the experiments of Fig. 1.

ammonium chloride, which is known to increase the rate of protein synthesis largely via its effects on intracellular pH¹⁹. Moreover, if the egg cytoplasm is made less alkaline after treatment with TPA by adding acetate, a weak acid¹⁹, to the sea water in which the eggs are suspended, then the rate of protein synthesis falls back to the rate seen in the untreated egg. Finally, Table 1 shows that the TPA-induced increase in protein synthesis is prevented by the presence of 100 µM dimethylamiloride or by the removal of sodium ions from the sea water in which the eggs are suspended. These data indicate that the TPA-induced increase in intracellular pH is a necessary stimulus for protein synthesis.

Our results strongly suggest that TPA stimulates protein synthesis in unfertilized sea urchin eggs by activating a Na/H exchange at the oolemma, which in turn leads to a cytoplasmic alkalinization similar in magnitude to that observed at fertilization. We believe that these effects occur without the participation of a calcium transient of the magnitude observed at fertilization, though we cannot exclude the possibility that small alterations in cytoplasmic calcium occur. The absence of a calcium transient in TPA-treated eggs accounts for the disparity between the rates of protein synthesis in fertilized and TPA-treated eggs: it has been shown that maximal rates of protein synthesis are achieved only if both the calcium concentration and intracellular pH increase²³. The prevailing idea is that many of the actions of TPA may be explained by its ability to promote phosphorylation of specific membrane proteins through activation of a membrane protein kinase, protein kinase C^{9,24}. Protein kinase C is thought to be regulated *in vivo* by diacylglycerol, one of the two putative second messengers generated by the hydrolysis of plasma membrane polyphosphoinositides²⁵. We have shown that the other second messenger, inositol trisphosphate, will activate eggs and that physiological calcium concentrations promote the hydrolysis of egg membrane polyphosphoinositides²⁶. We suggest that diacylglycerol may be the second messenger that links the stimulation of the Na/H exchange to the calcium transient of the egg at fertilization and so promotes mitosis and cell division.

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Note added in proof: A stimulation of Na/H exchange by phorbol ester and diacylglycerol has recently been reported to occur in cells in culture²⁷.

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- Steinhardt, R. A. & Epel, D. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1915-1919 (1974).
- Whitaker, M. J. & Steinhardt, R. A. *Cell* **25**, 95-103 (1981).
- Whitaker, M. J. & Steinhardt, R. A. *Q. Rev. Biophys.* **15**, 593-666 (1982).
- Epel, D., Steinhardt, R. A., Humphreys, T. & Mazia, D. *Dev. Biol.* **40**, 245-255 (1974).
- Johnson, J. D., Epel, D. & Paul, M. *Nature* **262**, 661-664 (1976).
- Cuthbert, A. & Cuthbert, A. W. *Expl. Cell Res.* **114**, 409-415 (1978).
- Payan, P., Girard, J.-P. & Ciapa, B. *Dev. Biol.* **100**, 29-38 (1983).
- Nishizuka, Y. *Phil. Trans. R. Soc. B302*, 101-112 (1983).
- Castagna, M. *et al. J. biol. Chem.* **257**, 7847-7851 (1982).
- Dicker, P. & Rosengurt, E. *Biochem. biophys. Res. Commun.* **100**, 433-441 (1981).
- Besterman, J. M. & Cuatrecasas, P. *J. Cell Biol.* **99**, 340-343 (1984).
- Whiteley, B., Cassel, D., Zhuang, Y.-X. & Glaser, L. *J. Cell Biol.* **99**, 1162-1166 (1984).
- Timourian, H. & Uno, J. *Expl. Cell Res.* **48**, 173-176 (1967).
- Hecker, E. (ed.) *Carcinogenesis and Biological Effects of Tumour Promoters* (Raven, New York, 1982).
- Steinhardt, R. A., Zucker, R. & Schatten, G. *Dev. Biol.* **58**, 185-196 (1977).
- Zucker, R. S., Steinhardt, R. A. & Winkler, M. M. *Dev. Biol.* **68**, 285-295 (1978).
- Baker, P. F. & Whitaker, M. J. *Nature* **276**, 513-515 (1978).
- Vacquier, V. D. & Brandriff, B. *Dev. Biol.* **47**, 12-31 (1975).
- Winkler, M. M. & Grainger, J. L. *Nature* **273**, 236-248 (1978).
- Thomas, J. A., Buchsbaum, R. N., Zimniak, A. & Racker, E. *Biochemistry* **18**, 2210-2218 (1979).
- Vigne, P., Frelin, C., Cragoe, E. J. Jr & Lazdunski, M. *Molec. Pharmac.* **25**, 131-136 (1984).
- Shen, S. S. & Steinhardt, R. A. *Nature* **282**, 87-89 (1979).
- Winkler, M. M., Steinhardt, R. A., Grainger, J. L. & Minning, L. *Nature* **287**, 558-560 (1980).
- Nishizuka, Y. *Nature* **308**, 693-698 (1983).
- Berridge, M. J. *Biochem. J.* **220**, 345-360 (1984).
- Whitaker, M. J. & Irvine, R. F. *Nature* **312**, 636-639 (1984).
- Moolenaar, W. H., Tertoolen, L. G. J. & de Laat, S. W. *Nature* **312**, 371-374 (1984).

Focus formation in rat fibroblasts exposed to a tumour promoter after transfer of polyoma *plt* and *myc* oncogenes

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The gene encoding the large-T protein of polyoma virus (*plt*), the E1A genes of adenoviruses, the viral *myc* gene (*v-myc*) or rearranged forms of the cellular *c-myc* gene confer on rat embryo fibroblast (REF) cells in primary culture a series of new properties ('immortality', reduced serum requirement and sensitivity to transformation by viral and activated cellular oncogenes) but do not induce the appearance of transformed foci¹⁻⁶. We now report that focus formation can be induced after transfer of these genes into either REF or established FR3T3 rat cells by subsequent exposure to the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA)^{7,8}. Frequencies of transformation are in the same range as those usually observed for transformation with complete polyoma DNA or with a mixture of cloned *myc* and *ras* oncogenes. These results further characterize the 'immortalized' state induced by *plt* and *myc* as one in which the cells maintain a normal growth control in many respects but can be further acted upon to produce a neoplastic progeny.

Complete transformation of REF cells first requires the establishment of immortalization, a complex phenotype induced by *plt*, adenovirus E1A or *v-myc* and *c-myc* oncogenes (group I). The activity of these genes allows subsequent transformation by a second class of oncogenes (group II) which includes polyoma virus *pmt* (middle-T protein), adenovirus E1B, retroviral and activated cellular *ras* genes²⁻⁵. Transfer of *plt*, rearranged *myc* or adenovirus E1A genes into REF cells results in all cases in the same alterations of growth control in culture,

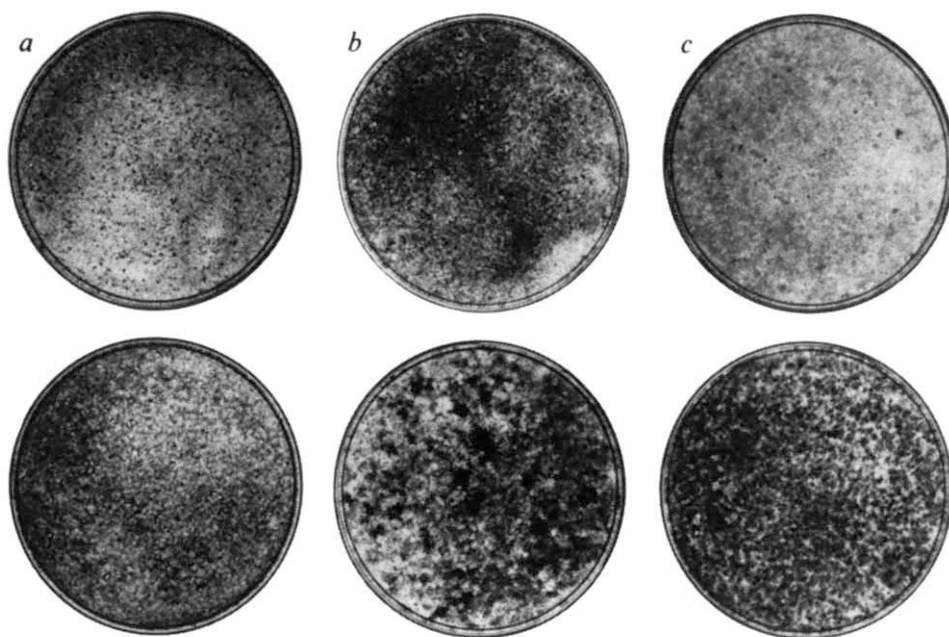


Fig. 1 Effect of TPA on FR3T3 cells immediately after transfer of the *plt* gene and on FR3T3-LT1 cells, which express constitutively the polyoma virus large-T protein¹. Experimental conditions are described in the text and in Table 1 legend. *a*, FR3T3; *b*, FR3T3 after transfer of plasmid pPyLT1; *c*, FR3T3-LT1. Upper row, control cultures treated with DMSO alone; bottom row, TPA-treated cultures.

most characteristically the acquisition of unlimited growth ability and reduced requirements for serum factors (refs 2, 6 and our unpublished results). Apart from these changes, immortalized cells retain the low saturation density and anchorage dependence of normal fibroblasts and thus cannot be selected by the usual transformation procedures (focus formation, growth in suspension). However, although still normal in many respects, *plt*- and *myc*-immortalized lines exhibit an increased probability of further oncogenic transformation. Tumour promoters transform cells which have first undergone an 'initiation' event^{7,8} induced by carcinogens, which have been shown independently to immortalize rodent embryo fibroblasts^{9,10}. We therefore investigated whether cells immortalized by *plt* or *myc* could be transformed further by treatment with TPA.

The effect of a 12-day exposure to TPA (2×10^{-5} M in 0.01% dimethyl sulphoxide, DMSO) after transfer of the polyoma *plt* gene was first studied in REF cells. Control cultures either were not treated at all or were treated after gene transfer with DMSO in the absence of TPA or were treated only with TPA. Foci developed in cultures treated with TPA after gene transfer (data not shown), but precise quantitation was hampered by the background of cells growing past confluency in primary REF cells, especially after treatment with TPA, which is known to

have mitogenic effects¹¹. Selection for colonies in agarose-gelified medium, however, produced unambiguous results (Table 1). Whereas neither treatment with TPA nor transfer of *plt* alone led to colony formation, the combination of the two treatments resulted in the appearance of clones that produced large colonies in suspension after the removal of TPA. Establishment of cell lines from such colonies (not shown) confirmed that they stably maintained their transformed phenotype. Transfer of the polyoma *pmt* gene (middle-T protein), with or without TPA treatment, conferred on REF cells only the ability to grow for a limited number of generations in suspension, as expected from the absence of an immortalization function.

We then investigated whether TPA produced the same effect in cells of the established line FR3T3. This would imply that the spontaneous immortalization event that gave rise to the line is not equivalent to immortalization by *plt* or *myc* genes, as has been suggested for FR3T3 rat cells⁶ and BALB/c3T3 mouse cells¹² by studies on the reduced requirement for serum growth factors in culture which is conferred by group I oncogenes. TPA treatment, in the same conditions as in the previous experiments, did not affect FR3T3 cell growth; however, following transfer of the *plt* gene, foci appeared within 4–6 weeks of TPA removal (Fig. 1). The same result was obtained when *plt* was replaced by the viral *myc* gene or by a rearranged mouse *c-myc* gene (carried on plasmids pSV-v-myc and pSV-c-myc-1, respectively⁴; Table 2). The numbers of foci per plate after *plt* and TPA treatment were in the same range as obtained after transfer of

Table 1 Colony formation in agarose medium by REF cells after transfer of isolated polyoma genes and subsequent treatment with TPA

Transfer of plasmid	Viral protein	TPA treatment	No. of colonies per plate (2×10^5 cells)	Size of colonies
None	—	—	0, 0, 0	—
None	—	+	0, 0, 0	—
pPyLT1	Large-T	—	0, 0, 0	—
pPyLT1	Large-T	+	2, 5, 8, 1, 3, 10	$>10^4$ cells*
pPyMT1	Middle-T	+	21, 13, 10, 17, 25, 19	<50 cells

REF secondary cultures were prepared as described previously² and DNA of plasmids pPyLT1 (*plt* gene) and pPyMT1 (*pmt* gene)^{14,15} transferred by the protoplast fusion technique also as described elsewhere¹. Cells were maintained for a total of 12 days with three medium changes in DMEM supplemented with 10% newborn calf serum (Gibco) and containing either 2×10^{-5} M TPA (Sigma) in 0.01% DMSO or 0.01% DMSO without TPA. Cells were then trypsinized and seeded in agarose medium¹ at a density of 2×10^5 cells per plate (three to six identical plates). The number of colonies in each plate was counted 3 weeks later.

* Macroscopically visible colonies.

Table 2 Focus formation by FR3T3 cells after transfer of *plt* and *myc* genes and subsequent treatment with TPA

Transfer plasmid	TPA treatment	Foci per plate (2×10^5 cells)
None	—	0, 0, 0
None	+	0, 0, 0
pPyLT1	—	0, 0, 2
pPyLT1	+	7, 15, 18
pSV-v-myc	—	0, 0, 1
pSV-v-myc	+	3, 4, 9
pSV-c-myc-1	—	0, 0, 0
pSV-c-myc-1	+	16, 22, 24

DNA of plasmids pPyLT1¹⁵, pSV-v-myc and pSV-c-myc-1⁴ was transferred into FR3T3 cells by the protoplast fusion technique as described in ref. 1 and TPA treatment was applied as described in Table 1 legend. Foci were counted following Giemsa staining after 6 weeks.

Table 3 Stability of the transformed phenotype during prolonged growth in the absence of TPA in cell lines derived from foci induced by TPA after transfer of the *plt* and *myc* oncogenes

Cell line	Plating efficiency in agarose medium (%)
FR-PLTTPA-32	10
FR-CMYCTPA-3	14
FR-VMYCTPA-3	8
FR3T3	<0.001

Cell lines FR-PLTTPA-32, FR-VMYCTPA-3 and FR-CMYCTPA-3 were isolated after transfer of plasmids pPyLT1, pSV-v-myc and pSV-c-myc-1, respectively, and subsequent TPA treatment as described in Table 2. For focus formation, the cultures had been maintained in DMEM without TPA for 4 weeks. Cells picked from foci were then grown in the same medium for three successive passages (~10 generations), still in the absence of TPA, and eventually seeded in agarose-gelified DMEM (2×10^5 cells per plate), in parallel with control FR3T3 cells. The number of colonies per microscope field, counted after 1 week, is expressed as a percentage of the input.

a wild-type polyoma genome expressing both the *plt* and *pmt* genes (data not shown; see ref. 1). When cells picked from such foci were grown further in Dulbecco's modified Eagle's medium (DMEM) in the absence of TPA, they still exhibited the characteristic morphology of transformed cells, and their efficiency of plating in agarose medium (10–20%) was comparable with that of typical virus-transformed lines¹³ (Table 3). Cells that permanently expressed immortalizing oncogenes were also treated with TPA. Such cell lines had been derived previously from FR3T3 cells after transfer of *plt* and *myc* and selection by colony formation in medium supplemented only with 0.5% serum^{1,6}. Unlike the original FR3T3 cells, such cultures reacted to TPA treatment by a dramatic change in their growth properties, showing an increase in saturation density within 2 weeks of TPA removal (Fig. 1).

Expression of polyoma virus *plt*, *v-myc* and rearranged *c-myc* genes therefore not only confers on normal rodent fibroblasts a set of new growth properties, such as immortalization or serum independence, but also allows further transformation by activated *ras* and polyoma *pmt* genes^{1–6}; these genes bring the cells into a state where they now respond to environmental stimuli that do not induce permanent transformation of the original normal cell. Our results further demonstrate that the phenotype induced by group I oncogenes is not equivalent to that of the spontaneously immortalized 3T3 cells, as previously indicated by studies on growth factor requirements^{6,12}. From a practical viewpoint, we note that, as found previously using other biological assays^{6,12}, gene transfection combined with TPA treatment may provide a means of discovering new oncogenes of this group possibly activated in human and experimental tumours.

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- Rassoulzadegan, M. *et al.* *Nature* **300**, 713–718 (1982).
- Rassoulzadegan, M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 4354–4358 (1983).
- Van den Elsen, P. J., de Pater, S., Houweling, A., van der Veer, J. & van der Eb, A. *Gene* **18**, 175–185 (1982).
- Land, H., Parada, L. F. & Weinberg, R. A. *Nature* **304**, 596–602 (1983).
- Ruley, H. E. *Nature* **304**, 602–606 (1983).
- Mouneau, E., Lemieux, M., Rassoulzadegan, M. & Cuzin, F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5758–5762 (1984).
- Beremblum, I. *Cancer Res.* **1**, 44–48 (1941).
- Heidelberg, C. A. *Rev. Biochem.* **44**, 79–111 (1975).
- Newbold, R. F. & Overell, R. W. *Nature* **304**, 648–651 (1983).
- Newbold, R. F., Overell, R. W. & Connell, J. R. *Nature* **299**, 633–635 (1982).
- Weinstein, B., Lee, L. S., Fisher, P. B., Mufson, A. & Yamasaki, H. *J. supramolec. Struct.* **12**, 195–208 (1979).
- Armelin, H. A. *et al.* *Nature* **311**, 655–660 (1984).
- Rassoulzadegan, M., Perbal, B. & Cuzin, F. *J. Virol.* **28**, 1–5 (1978).
- Tyndall, C., La Mantia, G., Thacker, C. M., Favaloro, J. & Kamen, R. *Nucleic Acids Res.* **9**, 6231–6250 (1981).
- Treisman, R., Novak, U., Favaloro, J. & Kamen, R. *Nature* **292**, 595–600 (1981).

Independent effects of growth hormone releasing factor on growth hormone release and gene transcription

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The synthesis and secretion of growth hormone (GH) by the somatotrophic cells of the anterior pituitary is under complex hormonal regulation. The hypothalamic peptides, growth hormone releasing factor (GHRF) and somatostatin, respectively stimulate^{1–3} and block⁴ GH release. We have shown that treatment of primary cultures of rat anterior pituitary cells with GHRF stimulates transcription of the GH gene, as well as GH release, and that this reflects the response of pituitary cells *in vivo*⁵. Glick *et al.* have shown a stimulation of GH mRNA levels in normal pituitary cells in response to GHRF⁶. Because normal pituitary cells are the physiological target for GHRF and because they exhibit both transcriptional and release responses to the hormone, they provide a valuable system with which to examine the important possibility of a link between hormone synthesis and release in secretory cells. Using this system, we demonstrate here that GHRF stimulates GH gene transcription independently of GH release and, conversely, that other agents can stimulate GH release without affecting transcription of the GH gene.

Treatment of primary cultures of pituitary cells with GHRF leads to a rapid rise in intracellular levels of cyclic AMP⁷. This event, which has been previously shown to stimulate the release of GH from pituitary cells^{8–12}, suggests that the effects of GHRF are mediated, at least in part, by a cyclic AMP-dependent mechanism. GHRF- or cyclic AMP-stimulated GH release is blocked by agents that block voltage-dependent calcium channels, such as verapamil^{7–12}, demonstrating that the release of stored GH is calcium-dependent. Therefore, it seems that the sequence of events leading to GHRF-induced GH release includes both a rise in cyclic AMP levels and a Ca^{2+} -dependent step that causes GH release. Our experiments were designed to investigate the possible link between GHRF-stimulated GH release and transcription, by determining which, if any, of the events leading to GH release are also involved in stimulation of GH gene transcription. We measured GH-specific transcription and GH release, as well as intracellular cyclic AMP levels, for each treatment described. Cell treatments were performed for 1 h, at which time nuclei were collected, and GH-specific transcription was determined by nuclear run-off assay⁵. GH release and intracellular levels of cyclic AMP were monitored by radioimmunoassay (RIA).

To determine whether release of stored hormone is required for the transcriptional stimulation by GHRF, we used several treatments to block GH release. We performed experiments in media containing either 2.5 mM EGTA, to chelate Ca^{2+} , or 100 μ M verapamil, to block voltage-dependent Ca^{2+} channels. In both cases, GHRF induction of GH release was blocked, but stimulation of GH gene transcription and intracellular cyclic AMP levels were not diminished (Fig. 1). Treatment with 10 nM somatostatin lowered basal GH release as well as GHRF-stimulated GH release (Fig. 2). The rise in cyclic AMP was diminished by somatostatin, as expected⁷ (60% reduction), but there was no inhibitory effect on basal, or GHRF-stimulated, GH gene transcription. These results indicate that the transcriptional response to GHRF is not dependent on GH release or on extracellular Ca^{2+} .

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Although the above experiments show that GH release is not required for GHRF stimulation of GH gene transcription, they do not address the possibility that GH release alone can stimulate transcription of the GH gene. To examine this possibility directly, we used several different treatments to stimulate GH secretion. Depolarizing concentrations of K^+ have been shown to release GH from pituitary cells^{13,14}. A 1-h treatment with 58 mM K^+ caused GH release without affecting either intracel-

lular cyclic AMP levels or the rate of transcription of the GH gene (Fig. 3). Treatment of pituitary cells with the tumour promoter, phorbol myristate acetate (PMA)^{15,16}, caused GH release¹⁷, but failed to stimulate cyclic AMP production or GH gene transcription (Fig. 3). Forskolin, a diterpene compound

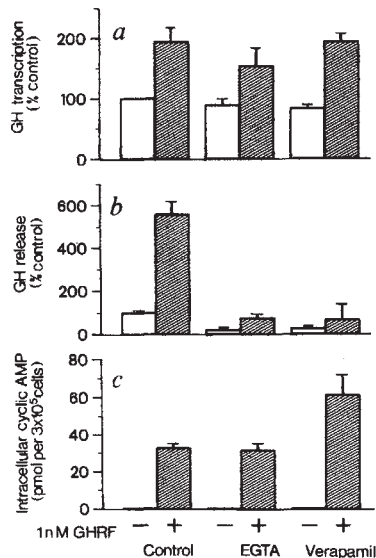


Fig. 1 Effects of EGTA and verapamil on GHRF stimulation of GH gene transcription (a), GH release (b), and intracellular cyclic AMP levels (c). Parallel plates of cells were treated with 1 nM GHRF (+) or GHRF dilution buffer (-). Where indicated, the medium contained 2.5 mM EGTA or 100 μ M verapamil. GH-specific transcription and GH release were determined from the same experiment and are expressed as % of control. Cyclic AMP was measured in a separate parallel experiment and is expressed as pmol cyclic AMP per 3×10^5 cells.

Methods. a, b, Primary cultures of anterior pituitary cells were prepared as described previously^{5,25,26} and plated at a density of 5×10^6 cells per 60-mm dish in medium containing 5 nM dexamethasone and 30 pM triiodothyronine. After 3 days in culture, the cells were washed by multiple medium change with B-PJ medium containing 0.1% bovine serum albumin and 2.5×10^{-4} M ascorbic acid²⁶ and returned to the incubator. After 1 h of incubation, they were treated with 1 nM rat hypothalamic GHRF (rhGHRF, synthesized by J. Rivier³) or a comparable volume of GHRF dilution buffer (1 mM acetic acid), in fresh B-PJ medium (control) or B-PJ containing 2.5 mM EGTA or 100 μ M verapamil. After 1 h of treatment, the medium was removed from the cells and assayed for immunoreactive GH by RIA⁷. Cells were lysed, nuclei were prepared and elongation of nascent RNA chains was carried out as previously described²⁷. RNA was prepared²⁷ from the nuclei and hybridized to excess plasmid DNA immobilized on nitrocellulose filters. The filters were washed, RNase-treated, dried and counted in toluene scintillation fluid. GH gene transcription was measured by quantifying RNase-resistant hybridization to a full-length GH cDNA clone. Background hybridization was determined by hybridization to pBR322 and the background values were subtracted from the GH hybridization values to give specific hybridization. GH-specific hybridization was determined as p.p.m. of total input RNA, and expressed as % of control (mock-treated). In reporting the data, no corrections were made for the size of the hybridization probe or the efficiency of hybridization. Each point represents treatment of a single plate of cultured cells and all experiments were repeated at least twice. Hybridizations were done in triplicate and data are presented as the mean $\pm$ s.e. for triplicate hybridizations from two similar experiments. c, Cells were prepared as in a, except that they were plated at 3×10^5 cells per well, in Linbro 24-well Petri dishes. Cell treatments were carried out as in a. After 1 h of treatment, cells were processed for cyclic AMP extraction as described previously²⁸. All treatments were carried out in triplicate wells. Cyclic AMP was assayed⁷ by RIA.

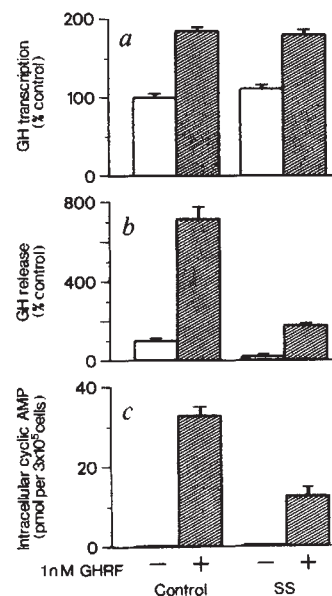


Fig. 2 Effect of somatostatin (SS) on GHRF stimulation of GH gene transcription (a), GH release (b), and intracellular cyclic AMP levels (c). Parallel plates of cells were treated with 1 nM GHRF (+) or dilution buffer (-), in the presence or absence of 10 nM SS in the medium.

Methods. Same as for Fig. 1. The experiment was repeated three times with similar results, and the data shown are from one representative experiment.

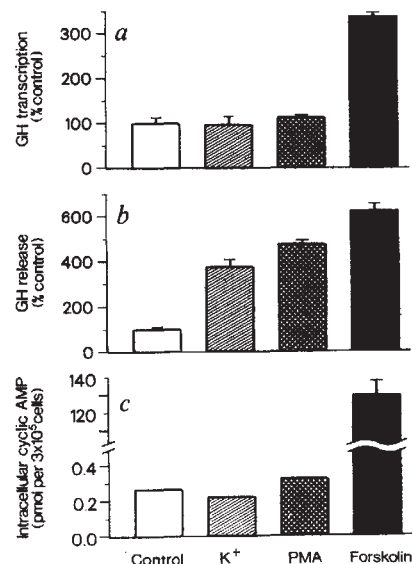


Fig. 3 Effects of K^+ , PMA and forskolin on GH gene transcription a, GH release b, and intracellular cyclic AMP levels c. Parallel plates of cells were incubated in the presence or absence of 58 mM K^+ , 100 nM PMA, or 10 μ M forskolin. In a and b, values for each treatment are expressed as per cent of the control value for that treatment. In c, absolute values are shown. Controls for c did not differ significantly.

Methods. Same as for Fig. 1, except that K^+ treatment was carried out in Krebs' Ringer's buffer with 11 mM glucose. Treatments were performed at least twice with similar results, and results reported are from one representative experiment. Where error bars are not shown, the standard error was too small to plot.

that activates the catalytic subunit of adenylate cyclase¹⁸, also stimulated GH release, but, unlike K⁺ and PMA, forskolin caused a rise in intracellular cyclic AMP levels and stimulated GH gene transcription also (Fig. 3).

Exposure of pituitary cells to GHRF provokes several cellular responses, including a rise in intracellular cyclic AMP levels and a Ca²⁺-dependent step that leads to GH release. We have shown that GH release is not the stimulus for the increase in transcription, by demonstrating that the transcriptional induction occurs in conditions that inhibit GH release, and that two treatments that stimulate GH release in the absence of GHRF have no effect on transcription of the GH gene. As GHRF stimulation of GH gene transcription is not dependent on external Ca²⁺, it also follows that the Ca²⁺-dependent step in the release response is not the stimulus for the transcriptional response.

As the physiological inhibitor of GHRF action, it might be expected that somatostatin would block the transcriptional effects of GHRF. Somatostatin reduces, but does not eliminate, the GHRF-stimulated rise in cyclic AMP⁷, and has been shown to activate the N_i (inhibitory) subunit of adenylate cyclase¹⁹; but there is much evidence to suggest that somatostatin exerts another inhibitory effect on GH release at the calcium-dependent step(s) in the release pathway, distal to the elevation of cyclic AMP levels. Somatostatin blocks stimulation of GH release by cyclic AMP derivatives²⁰ and agents that raise cyclic AMP levels^{7,11}, as well as treatments which cause influx of Ca²⁺ (refs 13, 14). Furthermore, Stachura has proposed that somatostatin has no effect on GH synthesis, based on the observation that dibutyl cyclic AMP causes a build-up of stored GH behind a somatostatin block^{21,22}. Our results show that somatostatin inhibits GH release without influencing GHRF-stimulated transcription of the GH gene, which is consistent with Stachura's model.

Of the agents we used to stimulate GH release in the absence of GHRF, forskolin caused a rise in cyclic AMP levels, whereas PMA and K⁺ did not. Of the three agents, only forskolin stimulates GH gene transcription. These data, together with our observation that neither EGTA, verapamil, nor somatostatin eliminates the GHRF-induced rise in cyclic AMP, suggest a link between the rise in intracellular cyclic AMP levels and stimulation of GH gene transcription. If cyclic AMP were responsible for the stimulation of GH gene transcription, such an effect might be mediated by phosphorylation of putative nuclear regulatory proteins. Phosphorylation of nuclear proteins has been associated with the stimulation of prolactin transcription by thyrotropin-releasing hormone²³ and cyclic AMP²⁴. In our system we have observed phosphorylation of H1 histone at serine 37 in response to treatment with GHRF or 8-bromo cyclic AMP (M.B. and M. Waterman, unpublished observations). Taken together, the data presented here clearly show that GHRF stimulation of GH gene transcription occurs independently of GH release and suggest a role for cyclic AMP as the mediator of the transcriptional stimulation.

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1. Rivier, J., Spiess, J., Thorner, M. & Vale, W. *Nature* **300**, 276–278 (1982).
2. Guillemin, R. *et al. Science* **218**, 585–587 (1982).
3. Spiess, J., Rivier, J. & Vale, W. *Nature* **303**, 532–535 (1983).
4. Brazeau, P. *et al. Science* **179**, 77–79 (1973).
5. Barinaga, M. *et al. Nature* **306**, 84–85 (1983).
6. Gick, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 1553–1555 (1984).

7. Bilezikjian, L. M. & Vale, W. W. *Endocrinology* **113**, 1726–1731 (1983).
8. Schofield, J. G. *Nature* **215**, 1382–1383 (1967).
9. Cooper, R. H., McPherson, M. & Schofield, J. G. *Biochem. J.* **127**, 143–154 (1972).
10. Sundberg, D. K., Fawcett, C. P. & McCann, S. M. *Proc. Soc. exp. Biol. Med.* **151**, 149–154 (1976).
11. Sheppard, M. S., Spence, J. W. & Kraicer, J. *Endocrinology* **105**, 261–268 (1979).
12. Spence, J. W., Sheppard, M. S. & Kraicer, J. *Endocrinology* **106**, 764–769 (1980).
13. Kraicer, J. & Spence, J. W. *Endocrinology* **108**, 651–657 (1981).
14. Kraicer, J. & Chow, A. E. H. *Endocrinology* **111**, 1173–1180 (1982).
15. Castagna, M. *et al. J. biol. Chem.* **257**, 7847–7851 (1982).
16. Nield, J. E., Kuhn, L. J. & Vandenbark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **80**, 36–40 (1983).
17. Smith, M. & Vale, W. *Endocrinology* **107**, 1425–1431 (1980).
18. Seamon, K. B. & Daly, J. W. *J. Cyclic Nucleotide Res.* **7**, 201–224 (1981).
19. Jakobs, K. H., Aktories, K. & Schultz, G. *Nature* **303**, 177–178 (1983).
20. Vale, W. *et al. C. r. heb. Séanc. Acad. Sci., Paris Ser. D* **275**, 2913–2916 (1972).
21. Stachura, M. *Endocrinology* **101**, 1044–1053 (1977).
22. Stachura, M. *Endocrinology* **108**, 1027–1034 (1981).
23. Murdoch, G. H., Franco, R., Evans, R. M. & Rosenfeld, M. G. *J. biol. Chem.* **258**, 15329–15335 (1983).
24. Murdoch, G. H., Rosenfeld, M. G. & Evans, R. M. *Science* **218**, 1315–1317 (1982).
25. Vale, W., Grant, G., Amoss, M., Blackwill, R. & Guillemin, R. *Endocrinology* **91**, 562–572 (1972).
26. Vale, W., Vaughan, J., Yamamoto, G., Spiess, J. & Rivier, J. *Endocrinology* **112**, 1553–1555 (1983).
27. Evans, R. M., Birnberg, N. C. & Rosenfeld, M. G. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7659–7663 (1982).
28. Bilezikjian, L. M. & Vale, W. W. *Endocrinology* **113**, 657–662 (1983).

Growth induced by pulsatile infusion of an amidated fragment of human growth hormone releasing factor in normal and GHRF-deficient rats

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The discovery of human pancreatic growth hormone releasing factors (GHRFs)^{1,2} and subsequent characterization of human hypothalamic GHRF³ has led to studies on the role of these peptides in stimulating growth hormone (GH) release^{4–6}, and attempts to use GHRF peptides to increase growth rates in short children are already underway⁷. However, there is no experimental evidence in animals that exogenous GHRF promotes growth *in vivo*. Although anaesthetized rats release GH reproducibly in response to GHRF injections^{4,8}, the responses in conscious male rats are much more variable^{5,6}, perhaps because of their highly episodic endogenous GH secretory pattern⁹. In contrast, female rats secrete GH in a more continuous pattern¹⁰ and respond reproducibly to repeated injections of GHRF. We report here that it is possible to establish a 'male' type of GH secretory pattern in normal female rats by long-term pulsatile intravenous (i.v.) infusions of the active human GHRF fragment GHRF (1–29)NH₂. We found that this treatment accelerates growth and increases pituitary GH content, whereas continuous infusions of this GHRF fragment at the same daily dose are ineffective. Pulsatile, but not continuous GHRF also stimulates growth in animals made GHRF-deficient by neonatal monosodium glutamate treatment. Thus exogenous GHRF will stimulate growth in both GHRF-deficient and normal animals provided it is administered in an appropriate pattern.

Injections of hGHRF(1–29)NH₂ (1 µg) were given i.v. every 3 h to a group of six conscious female rats (Fig. 1a). This treatment produced plasma GH profiles in female rats which were very similar to the endogenous GH secretory pattern in male rats (Fig. 1b). There was no evidence of endogenous secretory episodes between the exogenously induced GH pulses, and all the animals became entrained, responding uniformly to successive injections with peak plasma GH levels exceeding 1 µg ml⁻¹. Using a chronic i.v. infusion system with programmable multi-channel infusion pumps^{11,12}, groups of eight female rats were given saline or hGHRF(1–29)NH₂ either as a continuous infusion (8 µg per day) or as 1-µg pulses every 3 h for 12 days. The ability of this long-term pulsatile GHRF treatment to elicit pulses of GH secretion was assessed by withdrawing

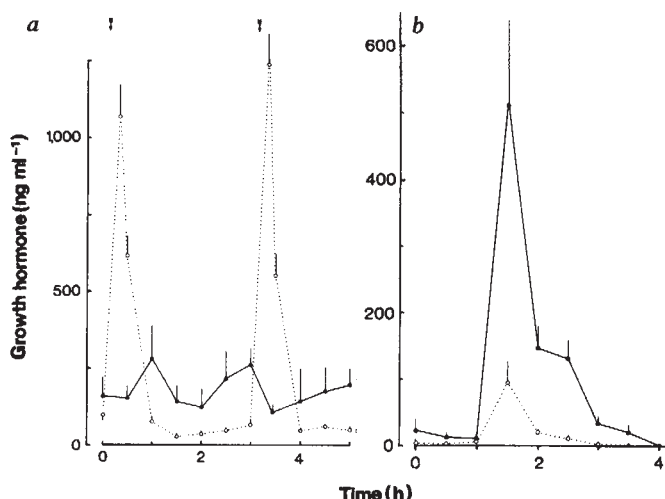


Fig. 1 Effects of hGHRF(1-29)NH₂ (a) and monosodium glutamate (b) on plasma GH levels. a, Six normal female rats (dotted line) received 1-μg injections of hGHRF(1-29)NH₂ (Bachem, UK, or Kabi-Vitrum, Sweden) via the i.v. cannulae at 9 a.m., 12 a.m., 3 p.m. and 6 p.m. Before and after the third and fourth injections (arrows), blood samples (100 μl) were withdrawn and the plasma separated and frozen. A second group of four female rats were given injections of saline to serve as controls (solid line). b, Newborn male rat pups received five subcutaneous injections of MSG (4 mg per g body weight) every second day from birth. Littermate controls received 10% saline. When 8 weeks old, the animals were catheterized as in a for blood sampling. Five MSG-treated rats (open circles) showed endogenous GH pulses which were blunted compared with those occurring in four normal male rats (solid circles). As endogenous GH pulses are not synchronized with respect to clock time, the peak concentrations for each individual rat have been aligned in this figure.

Methods. Sprague-Dawley rats (150–180 g) were housed individually in metabolic cages in a light- and temperature-controlled room and fed a powdered diet and distilled water *ad libitum*. Animals were anaesthetized with halothane/N₂O/O₂, and a silicone rubber catheter was inserted into the right jugular vein and exteriorized dorsally. Daily weight and food intake measurements showed that animals quickly resumed their normal growth. Sequential blood samples were obtained from conscious animals via extension cannulae attached to the catheters. Plasma GH levels were determined by RIA on 5- and 20-μl plasma samples using reagents supplied by NIADDK.

blood samples from three of the rats 5, 10 and 20 min after the 50th pulse of hGHRF(1-29)NH₂; the plasma GH values (mean ± s.e.m.) were 3,595 ± 1,400, 910 ± 155 and 326 ± 68 ng ml⁻¹, respectively. Pulsatile GHRF treatment produced a 30% increase in body weight gain compared with saline-infused controls, whereas the same total daily dose of hGHRF(1-29)NH₂ given as a continuous infusion for 12 days had no significant effect (Fig. 2a). A similar stimulating effect of pulsatile hGHRF(1-29)NH₂ on proximal tibial growth¹³ was observed in the same groups of animals (controls 110 ± 5; continuous GHRF 103 ± 8; pulsatile GHRF 117 ± 2 μm per day, mean ± s.e.m.), although the difference between continuous and pulsatile GHRF treatment was less clearly established ($P = 0.056$). At the end of the experiment, the anterior pituitary glands were extracted and assayed for GH content (Table 1a). Pulsatile hGHRF(1-29)NH₂ treatment significantly increased the pituitary content of GH, whereas continuous infusions were without effect.

We also compared continuous and pulsatile administration of hGHRF(1-29)NH₂ in animals made deficient in endogenous GHRF by neonatal exposure to monosodium glutamate (MSG)¹⁴. This treatment produces stunted growth¹⁵ and blunts endogenous GH pulses (Fig. 1b). However, MSG-treated rats can secrete large amounts of GH in response to exogenous GHRF. In five conscious MSG-treated male rats implanted with

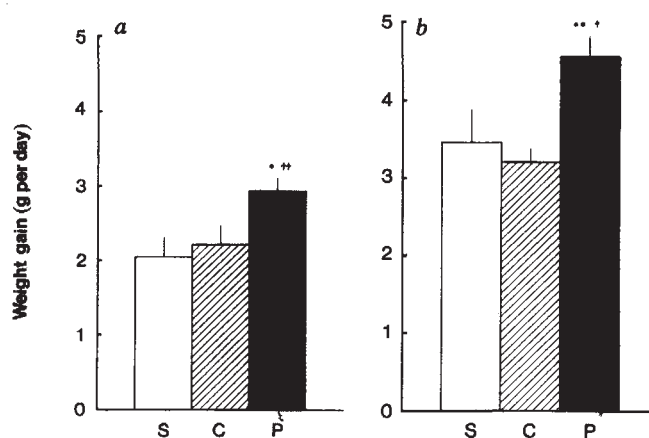


Fig. 2 Effects of continuous and pulsatile administration of hGHRF(1-29)NH₂ on growth in normal female (a) and MSG-treated male (b) rats. Values shown are means ± s.e.m. In both experiments, analysis of variance showed significant differences between the three treatment groups (P, pulsatile hGHRF(1-29)NH₂, solid bars; C, continuous hGHRF(1-29)NH₂, hatched bars; S, saline infused controls, open bars). In female animals (a), pulsatile GHRF produced significantly greater weight gains than either continuous GHRF (* $P < 0.05$) or saline treatment (†† $P < 0.025$). In MSG-treated male rats (b), pulsatile GHRF again was most effective († $P < 0.05$ vs. saline, and ** $P < 0.005$ vs. continuous GHRF).

Methods. Sprague-Dawley rats (150–180 g) were anaesthetized, a silicone cannula was placed in the right jugular vein, a head plate screwed to the occipital bone and the cannula passed through a protective spring to a fluid-tight swivel joint connected to multi-channel peristaltic infusion pumps^{11,12}. One pump ran continuously (1.6 ml per day) whereas a second pump delivered the same daily volume as a series of 200-μl pulses (2 min duration, every 3 h). The vehicle was phosphate-buffered saline (Dulbecco's 'A') containing 0.01% rat albumin and heparin (5 U ml⁻¹). The animals were weighed daily, which entailed disconnecting the continuous infusion for less than 2 min. Growth rates (g per day) were calculated by regressing the increase in body weight against time for each animal.

indwelling jugular cannulae, plasma GH values rose from 33 ± 7 ng ml⁻¹ to 2,640 ± 1,070 and 131 ± 26 ng ml⁻¹, respectively, 10 and 30 min after a single i.v. injection of 1 μg hGHRF(1-29)NH₂. Using the programmable i.v. infusion system, groups of MSG-treated male rats were given saline or hGHRF(1-29)NH₂, either continuously (8 μg per day) or in a pulsatile pattern (1-μg pulses every 3 h) for 10 days. As in normal female rats, pulsatile hGHRF(1-29)NH₂ stimulated body weight gain whereas continuous infusions of this peptide did not increase weight gain (Fig. 2b). Pulsatile hGHRF(1-29)NH₂ also produced a significant increase in long bone and tail growth in these MSG-treated rats (Table 2).

Although neonatal exposure to MSG results in smaller anterior pituitary glands and lower GH content in the adult, the pituitary GH concentration remains unchanged¹⁶. Unlike in normal female rats, we could show no trophic effects of hGHRF(1-29)NH₂ on pituitary GH content in MSG-treated animals (Table 1b). GHRF can exert a trophic effect *in vitro* on GH messenger RNA transcription¹⁷ in addition to its powerful secretagogue effect. However, multiple injections of GHRF do not increase the GH content of pituitary tissue autotransplanted to the kidney capsule¹⁸. As MSG disrupts other hypothalamic neurotransmitter systems¹⁹ in addition to GHRF neurones¹⁴, it is possible that the trophic effect of GHRF *in vivo* requires an intact hypothalamo-hypophyseal system.

These experiments are the first to show that a GHRF peptide can induce growth in both normal female and GHRF-deficient animals, and that the amount of growth depends on the pattern of administration. The female rat has been relatively neglected in studies of GHRF *in vivo* and offers considerable advantages

Table 1 Effect of hGHRF(1-29)NH₂ on anterior pituitary stores of GH

	(n)	Wet weight (mg)	Anterior pituitary GH content (µg)	GH concentration (µg per mg wet wt)
a Normal females				
Saline	(8)	9.8 ± 0.3	613 ± 36	62 ± 3
Continuous GHRF	(8)	10.2 ± 0.7	623 ± 68	61 ± 5
Pulsatile GHRF	(8)	10.3 ± 0.4	834 ± 75*	81 ± 7*
b MSG-treated males				
Saline	(8)	4.5 ± 0.5	522 ± 119	104 ± 17
Continuous GHRF	(8)	4.6 ± 0.5	577 ± 112	115 ± 14
Pulsatile GHRF	(8)	5.3 ± 0.6	680 ± 161	115 ± 20

Anterior pituitary glands were removed, weighed, homogenized individually in 1 ml phosphate-buffered saline pH 7.4, centrifuged for 10 min at 13,000g and the supernatants assayed for rat GH by radioimmunoassay (RIA), using six doubling dilutions in duplicate. See text for an explanation of the different treatment groups. In female rats (a), pulsatile hGHRF(1-29)NH₂ for 12 days increased pituitary GH content and concentration. In male MSG-treated rats (b) hGHRF(1-29)NH₂ had no significant effect on pituitary GH.

* $P < 0.05$ vs. both saline and continuous GHRF.

of uniformity and availability, together with an intact hypothalamo-hypophyseal system. The possibility that prolonged pulsatile GHRF treatment might alter reproductive cycles in the female rat, and thus indirectly influence body growth must be considered, although this explanation is unlikely to account for the stimulation of growth produced in male rats by pulsatile GHRF. The simplest explanation for our results is that effective GHRF levels in the hypophyseal portal vessels are achieved only by giving the peptide in pulses. Continuous infusions of GHRF require higher total dose rates to stimulate GH release and can result in rapid depletion of pituitary GH stores²⁰. In the case of MSG-treated animals, we are replacing the depleted GHRF secretion, whereas in normal female rats we are presumably overriding endogenous GHRF secretion. Whether or not the endogenous secretory profile of GHRF in male rats shows 3-hourly episodes, an exogenous GHRF peptide given in 3-hourly pulses produces a plasma GH profile which mimics the normal male pattern. We have previously shown in hypophysectomized male rats that the growth responses to a given dose of GH are greater if the GH is given in 3-hourly pulses¹². The pattern of GH secretion is less pulsatile in female rats, and this may partly underlie their slower growth rates²¹. We reasoned that maintaining a male pattern of GH secretion might increase the growth rate of normal females towards that of the male rat. This proved to be the case.

Both normal female rats and MSG-treated male rats may provide useful data when considering the treatment of GHRF-deficient children with pulsatile GHRF⁷, especially where this has to be given by the subcutaneous route, which inevitably reduces the pulsatility. Our demonstration that a more effective GH secretory profile can be imposed in normal, intact (female) animals by patterned exogenous GHRF may well have applica-

bility in animal husbandry, provided suitable simple means for achieving the optimal pattern of administration can be found. The obvious corollary of this result is that a sub-optimal infusion regime might impose a less effective growth rate, which could conceivably be useful therapeutically in children with excessive rates of growth. At its simplest level, the chronically cannulated female rat offers a convenient model in which to test the effects of different routes and patterns of administration of GHRF, its analogues and inhibitors on whole body growth.

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- Rivier, J., Spiess, J., Thorner, M. & Vale, W. *Nature* **300**, 276-278 (1982).
- Guillemin, R. *et al. Science* **218**, 585-587 (1982).
- Ling, N. *et al. Proc. natn. acad. Sci. U.S.A.* **81**, 4302-4306 (1984).
- Wehrenberg, W. B. *et al. Biochem. biophys. Res. Commun.* **109**, 382-387 (1982).
- Wehrenberg, W. B. *et al. Endocrinology* **111**, 2147-2148 (1982).
- Wehrenberg, W. B. *et al. Neuroendocrinology* **36**, 489-491 (1983).
- Thorner, M. O. *et al. in Proc 7th Int. Congr. Endocr. Abstr S. 167* (Excerpta Medica, Amsterdam, 1984).
- Wehrenberg, W. B., Baird, A. & Ling, N. *Science* **221**, 556-558 (1983).
- Tannenbaum, G. S. & Martin, J. B. *Endocrinology* **98**, 562-570 (1976).
- Eden, S. *Endocrinology* **105**, 555-560 (1979).
- Clark, R. G. & Robinson, I. C. A. F. *J. Physiol., Lond.* **350**, 2P (1984).
- Clark, R. G., Jansson, J. O., Isaksson, O. & Robinson, I. C. A. F. *J. Endocr.* **104**, 53-61 (1985).
- Hansson, L. I., Menander-Sellman, K., Stenstrom, A. & Thorngren, K.-G. *Calcif. Tissue Res.* **10**, 238-251 (1972).
- Bloch, B. *et al. Nature* **307**, 272-273 (1984).
- Olney, J. W. *Science* **164**, 719-721 (1969).
- Millard, W. J., Martin, J. B. Jr, Audet, J., Sagar, S. M. & Martin, J. B. *Endocrinology* **110**, 540-550 (1982).
- Barinaga, M. *et al. Nature* **306**, 84-86 (1983).
- Jansson, J. O., Carlsson, L. & Isaksson, O. *Endocrinology* (in the press).
- Jennes, L., Stumpf, W. E., Bissette, G. & Nemeroff, C. B. *Brain Res.* **308**, 245-253 (1984).
- Wehrenberg, W. B., Brazeau, P., Ling, N., Textor, G. & Guillemin, R. *Endocrinology* **114**, 1613-1616 (1984).
- Jansson, J. O., Ekberg, S., Isaksson, O. & Eden, S. *Endocrinology* **114**, 1287-1294 (1984).
- Hughes, P. C. R. & Tanner, J. M. *J. Anat.* **106**, 349-370 (1970).

Table 2 Effect of hGHRF(1-29)NH₂ on tail and bone growth in MSG-treated rats

	(n)	Tail growth (mm)	Proximal tibial growth (µm per day)
Saline	(8)	15.4 ± 1.3	137 ± 12
Continuous	(8)	18.2 ± 2.6	137 ± 8
Pulsatile	(8)	20.6 ± 1.3*	159 ± 7†

Tail lengths of MSG-treated rats were measured²² under anaesthesia at the beginning and end of the infusion experiment (see text). The differences between these measurements are shown as mean ± s.e.m. Immediately before the infusions began, each rat received an injection of oxytetracycline (2 mg per 100 g body weight, i.v.). At the end of the experiment, the proximal tibias were dissected out and cumulative bone growth assessed by fluorescence microscopy according to the method of Hansson *et al.*¹³. Results were analysed using paired *t*-tests against littermate controls.

* $P < 0.001$ vs. saline; † $P < 0.05$ vs. continuous GHRF.

Tissue-specific expression of rat myosin light-chain 2 gene in transgenic mice

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One approach to determining how the differential expression of specific genes is regulated in higher organisms is to introduce cloned copies of the genes (or parts of the genes) into the genomes of individual organisms from the very beginning of their development. The way in which the exogenous genetic information behaves during the development of the experimental organisms can then provide a means of defining the DNA sequences that restrict the

expression of the gene to specific cell types and times of development. So far, several different genes have been introduced into the genomes of mice¹⁻¹⁵, but in only a few cases have the exogenous genes retained the tissue specificity of expression of the equivalent endogenous genes^{12,14,15}. I report here that in two out of three 'transgenic' mice carrying copies of the rat gene for skeletal muscle myosin light chain 2, the exogenous gene is expressed specifically in skeletal muscle cells. The sequences contained in the cloned copy of the myosin light-chain 2 gene used in these experiments are thus sufficient to confer a tissue-specific pattern of expression.

A recombinant plasmid (pRH3MLC)¹⁶ containing the rat skeletal muscle myosin light-chain 2 (MLC2) DNA sequences in their entirety, in a 4.7-kilobase (kb) DNA fragment, was cut with *Hind*III to release the rat DNA sequences from the plasmid DNA and used for injection into fertilized mouse eggs (Fig. 1a). Superovulated females of (BALB/c×C57BL/6J) F₁ were mated to males of (C57BL/6J×DBA) F₁. On the day after mating, fertilized eggs were recovered from the oviducts. The male pronuclei were microinjected with 1–2 pl containing ~500 molecules of the plasmid DNA. Eggs that survived the microinjection (~50%) were implanted into the oviducts of pseudopregnant CD-1 females for further development. Spleen DNA samples from 26 neonatal mice were analysed by Southern blot hybridization and four mice containing pRH3MLC sequences were identified. DNA isolated from spleens of these mice (designated MLC₁, MLC₂, MLC₃, MLC₄) and a control mouse was digested with *Hind*III, *Eco*RI or *Xba*I. Figure 1b shows an autoradiogram of a Southern blot of the fractionated DNA, hybridized to ³²P-labelled pRH3MLC DNA. In addition to the expected 4.7- and 4.3-kb DNA fragments in the *Hind*III digest, several minor fragments that hybridized to the probe could be

observed in DNA from mice MLC₁, MLC₂ and MLC₄, these fragments resulting from either junction fragments with cellular DNA or rearranged sequences.

As the pRH3MLC DNA was cut with *Hind*III before microinjection, multiple orientations of MLC2 and pBR322 DNA sequences were expected (Fig. 1). For example, in the *Eco*RI digests, the 1.6-kb DNA fragment seen in the four transgenic mice is a product of cleavage at the internal *Eco*RI sites of the MLC2 gene. The 2.0-kb DNA fragment in transgenic mice MLC₁, MLC₃ and MLC₄ is a product of cleavage at the 3' end *Eco*RI site of MLC2 gene, when it is integrated next to pBR322 DNA in a head-to-tail configuration. This fragment is undetectable in mouse MLC₂ DNA; instead a predominant 6.3-kb DNA fragment appears, indicating that most of the MLC2 DNA molecules in this mouse are arranged in tandem arrays with pBR322 DNA in a head-to-head orientation. Similarly, in the *Xba*I digests, the 3.9-kb DNA fragment is a product of cleavage at the two sites when pBR322 DNA is flanked on both sides with MLC2 DNA sequences. Analysis of the restriction pattern indicated that most of the copies of the injected DNA are integrated tandemly in the mouse genome. The number of pRH3MLC DNA copies in each mouse was estimated by comparing the intensity of the hybridized fragments with those of known amounts of plasmid DNA. Thus, mice MLC₁, MLC₂, MLC₃ and MLC₄ contain ~20, 10, <1 and 16 copies per cell, respectively.

To examine the transmission of the inserted DNA sequences to progeny, each of the four transgenic mice was mated with a normal mouse. Southern blot hybridization to spleen DNA from the progeny of MLC₁, MLC₂ and MLC₄ revealed that the inserted DNA sequences were transmitted faithfully through the germ line to ~50% of the progeny (data not shown; see Fig. 2), the expected frequency if the majority of the multiple MLC2 copies are integrated tandemly at a single site of one chromosome.

In contrast to the mendelian inheritance of pRH3MLC DNA sequences in mice MLC₁, MLC₂ and MLC₄, mouse MLC₃ did not transmit the injected DNA to any of its first 60 offspring. This female mouse contains <1 copy per cell of the inserted DNA (Fig. 1b) and is apparently a mosaic in which the injected DNA is present in only a small proportion of its somatic cells.

To investigate whether the expression of the rat MLC2 gene in transgenic mice was tissue-specific, RNA was extracted from skeletal muscle and other organs from the progeny of two transgenic mice. The presence of rat MLC2 transcripts in these RNA preparations was determined by the S₁ endonuclease technique, using as a probe the coding strand of the 630-base pair (bp) *Hinf*I/*Hinf*I DNA fragment derived from the 5' region of the rat MLC2 gene and labelled at the 3' end with reverse transcriptase (see Fig. 3). In stringent hybridization conditions

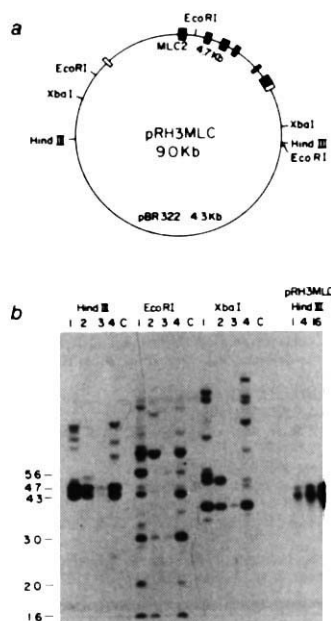


Fig. 1 Map of plasmid DNA pRH3MLC and Southern blot analysis of DNA from four transgenic mice. **a**, The 4.7-kb *Hind*III DNA fragment containing the rat MLC2 gene was cloned into the *Hind*III site of pBR322 (ref. 16). Boxes indicate the positions of the seven exons; empty boxes represent the 5'- and 3'-untranslated regions; solid boxes represent coding sequences. **b**, Total spleen DNA (10 µg) from the four transgenic mice (1–4) and a control mouse DNA (C) was digested with *Hind*III, *Eco*RI or *Xba*I. The DNA fragments were fractionated on a 0.8% agarose gel, transferred to nitrocellulose filter and hybridized with ³²P-labelled pRH3MLC DNA. The blot was washed in stringent conditions (0.1×SSC, 0.2% SDS at 75°C for 2 h) not allowing detection of the mouse gene. Plasmid pRH3MLC DNA samples digested with *Hind*III corresponding to 1, 4 and 16 copies per diploid mouse genome were included to estimate the copy number of inserted DNA in transgenic mice. Molecular sizes are shown in kb on the left.

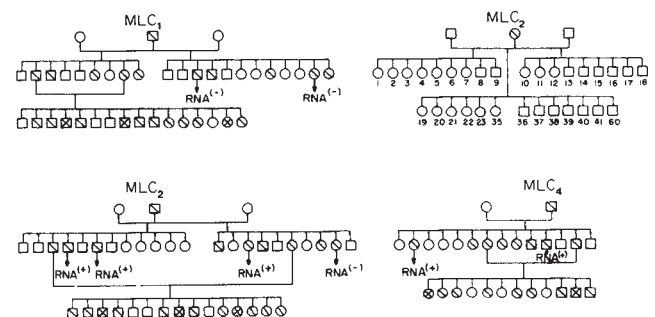


Fig. 2 Inheritance of pRH3MLC sequences in the four transgenic mice. Each transgenic mouse was mated with normal mouse. Heterozygous male and female progeny of MLC₁ and MLC₂ mice were mated to give rise to normal homozygous mice. Mouse MLC₃ is probably a mosaic. Squares represent males, circles females, intersecting diagonals homozygous mice, single diagonals heterozygous mice. Mice denoted RNA⁽⁺⁾ contained MLC2 transcripts in skeletal muscle whereas mice denoted RNA⁽⁻⁾ did not.

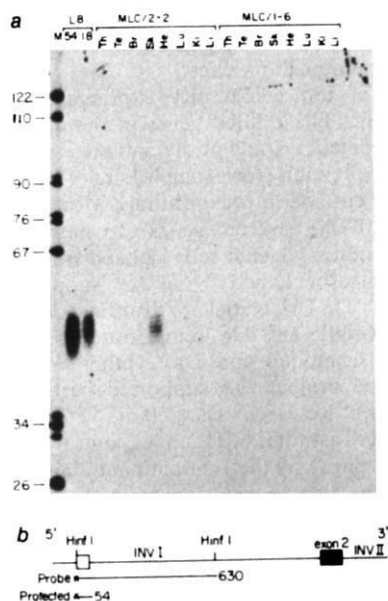


Fig. 3 S_1 analysis of rat MLC2 RNA in the progeny of transgenic mice MLC₁ and MLC₂. **a**, 25 μ g total RNA from the indicated tissues and total RNA from differentiated cultures of the myogenic cell line L8 were hybridized at 46 °C for 16 h to a 3'-end-labelled *Hinf*I/*Hinf*I DNA fragment prepared from pRH3MLC DNA (see scheme in **b**). Th, thymus; Te, testes; Br, brain; Sk, skeletal muscle; He, cardiac muscle; Lu, lung; Ki, kidney; Li, liver. Hybridization reactions were incubated with 5,000 U S_1 nuclease (Miles) for 30 min at 42 °C. S_1 -resistant hybrids were sized on 5% acrylamide/7M urea gel. **b**, Map of the 5' region of the rat MLC2 gene and the hybridization probe. Boxes, exons; solid lines, introns and 5'-flanking region; open box, 5'-untranslated exon; solid box, first coding exon. The 3' label is indicated by an asterisk and the lengths of the probe and the protected fragment are also indicated.

(hybridization at 46 °C and S_1 digestion at 42 °C), cross-hybridization to the endogenous mouse MLC2 transcripts did not occur. Authentic rat transcripts protect a 54-nucleotide DNA fragment from S_1 digestion. The appearance of several bands at that region probably results from S_1 nibbling at the end. Figure 3 shows that no rat MLC2 transcripts could be detected in skeletal muscle RNA or any of the other seven tissues of mouse MLC₁. In mouse MLC₂, however, transcripts were detected in skeletal muscle RNA but not in RNA from the other tissues tested. Analysis of RNA prepared from mouse MLC₄ revealed the presence of a large amount of the rat transcripts only in skeletal muscle (data not shown).

Muscle-specific transcripts in transgenic mice MLC₂ and MLC₄ could arise either from readthrough transcription initiated from upstream mouse promoter expressed in skeletal muscle or from initiation at the authentic rat MLC2 promoter. To distinguish between these two possibilities, the site of the initiation of these transcripts was determined. The coding strand of a 289-bp *Sau*3A/*Eco*RI DNA fragment ³²P-labelled with T4 polynucleotide kinase was hybridized to RNA from differentiated cultures of the rat myogenic cell line L8, to control mouse skeletal muscle RNA and to skeletal muscle RNA from males and females of the first generation progeny of mice MLC₁, MLC₂ and MLC₄. RNA from the rat myogenic cell line protected a 50-nucleotide DNA fragment (Fig. 4). The appearance of two bands spaced one nucleotide apart probably results from S_1 nibbling of the sequence that immediately precedes the cap site. RNA from control mouse skeletal muscle protected a 76-nucleotide DNA fragment. These unexpected results suggest that: (1) there is a similarity in the sequences of the 5'-untranslated exon and immediate flanking region of the rat and the mouse MLC2 genes and (2) the transcription of the mouse MLC2 gene is initiated ~20 nucleotides 5' to the initiation site of the rat MLC2 gene. In more stringent hybridization conditions, such as those

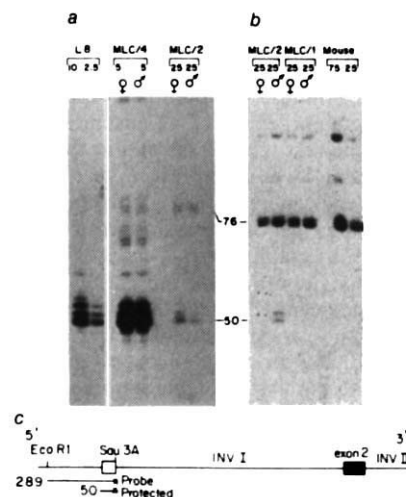


Fig. 4 The rat MLC2 gene is initiated correctly in progeny of transgenic mice MLC₂ and MLC₄. Total RNA from skeletal muscle of male (δ) and female (ϕ) progeny of transgenic mice MLC₁, MLC₂ and MLC₄, RNA from skeletal muscle of control mouse and from differentiated cultures of the myogenic cell line L8 were hybridized with 5'-end-labelled *Sau*3A/*Eco*RI DNA fragment (see **c**). RNA (μ g) in each hybridization reaction is indicated. **a**, Hybridization performed at 42 °C and S_1 digestion at 37 °C; **b**, hybridization at 37 °C and S_1 digestion at 32 °C. After treatment with S_1 nuclease, the resistant hybrids were sized on 5% acrylamide/7 M urea gels. **c**, Map of the 5' region of the rat MLC2 gene and the probe used in the hybridization. Boxes, exons; solid line, introns and 5'-flanking region; open box, 5'-untranslated exon; solid box, first coding exon. The 5' label is indicated by an asterisk and the lengths of the probe and the protected fragment are shown.

chosen for the *Hinf*I DNA fragment (Fig. 3), there was no cross-homology with the mouse exon. (Also, compare the intensity of the signals of the 76-nucleotide band in Fig. 4a, b.) The stringency was lowered here so that the hybridization to the mouse exon is an internal control for the intactness of the RNA preparations of mouse MLC₁ and the control mouse. RNA from three out of four progeny of transgenic mouse MLC₂ and two progeny of mouse MLC₄ protected the diagnostic 50-nucleotide DNA fragment (Fig. 4). As expected, skeletal muscle RNA of MLC₁ mice gave no detectable signal.

The MLC2 gene is a member of a large group of genes activated during terminal differentiation of muscle cells¹⁷. The results presented here demonstrate that in two out of the three transgenic mice tested, the injected rat MLC2 gene responded to normal developmental controls. Its expression was detected only in skeletal muscle. Thus, the 4.7-kb rat DNA fragment containing the entire MLC2 gene includes *cis*-acting sequences sufficient to specify the correct expression of this gene. RNA transcripts of this gene were found in skeletal muscle in three of four progeny of MLC₂ and in two progeny of MLC₄, and not in any other tissue. The RNA had the correct initiation site of the authentic rat MLC2 gene, which differs from the endogenous mouse MLC2 by ~26 nucleotides. Interestingly, a similar frequency of non-expressing progeny (two of eight) has been reported for transgenic mice expressing the rabbit β -globin gene in skeletal muscle¹.

The levels of expression of the inserted rat gene are ~5–10% (in MLC₂) and 5–10 $\times$ greater (in MLC₄) than in differentiated cultures of the rat myogenic cell line L8. The variable levels of specific expression could result from position effect, thus integration into non-homologous chromosomal sites may influence the level of expression of the foreign gene.

Several genes have been inserted into mice^{1–15}. Tissue-specific expression of introduced genes, however, has been reported

only in four other cases; the rearranged immunoglobulin in κ gene¹², the rearranged immunoglobulin heavy-chain gene¹⁵, the rat pancreatic elastase I gene¹⁴ and the fused gene consisting of the 5' end of the mouse β -globin gene spliced to the 3' end of the human β -globin gene (F. D. Constantini, personal communication). In the first three cases and in the rat MLC2 gene in mouse MLC₄, the level of expression was similar to that of the endogenous gene, whereas in the globin and rat MLC2 genes in mouse MLC₂, the level of expression was considerably lower than the endogenous counterparts. One possible interpretation is that the immunoglobulin and the elastase I genes include tissue-specific enhancer sequences that are recognized as such even when the genes are integrated at different chromosomal sites. Evidence for the dramatic effects of enhancer sequences on the efficiency of expression of genes introduced into somatic cells has been obtained¹⁸. There is no evidence that the injected MLC2 DNA fragment includes specific enhancer elements. Therefore, the hundredfold difference in the level of expression in the two transgenic mice could result from either the absence of such elements or their weakness. Thus, in mouse MLC₂, these

sequences are neutralized easily by the local chromosomal environment at the site of integration, whereas in mouse MLC₄ they overcome the position effect.

Production of transgenic mice expressing muscle-specific genes or specifically modified genes in the appropriate tissue would allow a detailed study of the parameters affecting tissue-specific gene expression (for example, site of integration, DNA methylation or chromatin organization). Moreover, the control of expression of the inserted genes can now be analysed in myogenic and non-myogenic cells isolated from these mice.

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1. Lacy, E., Roberts, S., Evans, E. P., Burtenshaw, M. D. & Costantini, F. D. *Cell* **34**, 343-358 (1983).
2. Gordon, J. W. & Ruddle, F. H. *Science* **213**, 1244-1246 (1981).
3. Wagner, E., Stewart, T. & Mintz, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5016-5020 (1981).
4. Wagner, T. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 6376-6380 (1981).
5. Costantini, F. & Lacy, E. *Nature* **294**, 92-94 (1981).
6. Brinster, R. L. *et al. Cell* **27**, 223-231 (1981).
7. Jahner, D. *et al. Nature* **298**, 623-628 (1982).
8. Palmiter, R. D. *et al. Nature* **300**, 611-615 (1982).

9. Burki, K. & Ullrich, A. *EMBO J.* **1**, 127-131 (1982).
10. Palmiter, R. D., Chen, H. Y. & Brinster, R. L. *Cell* **29**, 701-716 (1982).
11. McKnight, G. S., Hammer, R. E., Kuenzel, E. A. & Brinster, R. L. *Cell* **34**, 335-341 (1983).
12. Brinster, R. L. *et al. Nature* **306**, 332-336 (1983).
13. Stewart, T. A., Pattengale, P. K. & Leder, P. *Cell* **38**, 627-637 (1984).
14. Swift, C. H., Hammer, R. E., MacDonald, R. J. & Brinster, R. L. *Cell* **38**, 639-646 (1984).
15. Grosschedl, R., Weaver, D., Baltimore, D. & Costantini, F. *Cell* **38**, 647-658 (1984).
16. Nudel, U., Calvo, J., Shani, M. & Levy, Z. *Nucleic Acids Res.* **12**, 7175-7186 (1984).
17. Carmon, Y., Czosnek, H., Nudel, U., Shani, M. & Yaffe, D. *Nucleic Acids Res.* **10**, 3085-3097 (1982).
18. Gruss, P. *DNA* **3**, 1-5 (1984).

Regulation of a collagen gene promoter by the product of viral *mos* oncogene

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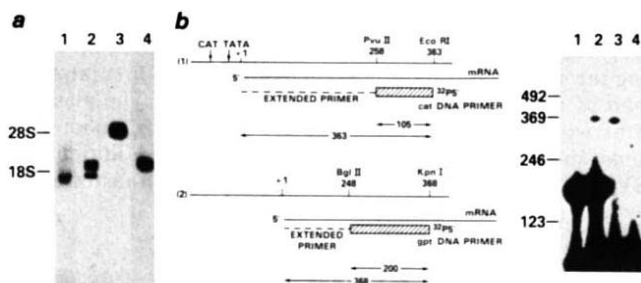
Oncogenic transformation of cells produces important changes in the biosynthetic pattern of certain cellular proteins¹⁻⁶. For example, the synthesis of type I collagen in transformed fibroblasts is severely reduced as a result of changes in transcription⁷⁻¹⁰. Here we report the results of DNA-mediated transfection experiments using recombinant plasmids in which the promoter region of the $\alpha 2(I)$ collagen gene is fused to an easily recognizable marker gene, and cell lines expressing the marker gene are isolated. Our data show that the expression of the marker gene fused to the cloned $\alpha 2(I)$ collagen promoter is strongly inhibited by *v-mos* transformation, suggesting that a common mechanism inhibits both the transfected and endogenous $\alpha 2(I)$ collagen promoters.

The $\alpha 2(I)$ collagen gene is very large (~40 kilobase pairs(bp) with ~50 exons)¹¹ and is therefore difficult to manipulate *in vitro*. To overcome this problem in studying its expression by DNA-mediated transfection experiments, we have fused the promoter region of this gene to either the bacterial gene for chloramphenicol acetyltransferase (*cat*)¹² or the gene for aminoglycoside phosphotransferase (*neo*)¹³. Although such constructions may not contain all the regulatory elements controlling the expression of the endogenous $\alpha 2(I)$ collagen gene, they do allow us to test whether the 5'-flanking sequences of the $\alpha 2(I)$ collagen gene which are present in these hybrid plasmids are sufficient for regulation by oncogenes. Figure 1 shows the plasmids constructed for our experiments. We first transfected NIH 3T3 cells with DNA of plasmid pHO1000, in which the chick $\alpha 2(I)$ collagen promoter is fused to the *cat* gene. This plasmid also contains the gene for guanine phosphoribosyltransferase

(*gpt*) fused to the early promoter of simian virus 40 (SV40). Clones positive for the expression of both the *gpt* and *cat* genes (AT1, AT2, AT3, etc.) were isolated. One of these clones (AT3) was examined by Southern blotting to show that the plasmid was stably integrated throughout at least two cycles of subcloning. We estimated that this cell line contains about eight copies of the $\alpha 2(I)$ collagen promoter-*cat* DNA unit (data not shown). A Northern hybridization experiment indicated that the RNA produced by the collagen promoter-*cat* transcription unit in these cells was a discrete species ~1,600 nucleotides (Fig. 2a), consistent with a transcript starting from within the cloned chicken $\alpha 2(I)$ collagen promoter and ending at the SV40 polyadenylation site present downstream from the *cat* gene in pHO1000. The results of a primer extension experiment (Fig. 2b) further indicate that the *cat* gene transcript starts at the correct site in the cloned $\alpha 2(I)$ collagen promoter. In the same cells, *gpt* transcripts initiate at the major transcription start site in the early SV40 promoter (Fig. 2b, lane 2).

To determine whether the expression of the hybrid $\alpha 2(I)$ collagen promoter-*cat* transcription unit was also inhibited by oncogenic transformation after it had been introduced into NIH 3T3, we first transformed three cell lines with the mouse Moloney sarcoma and leukaemia virus complex (MMSV). In all three clones, the level of CAT activity was reduced 7-14-fold after transformation (Fig. 3a, Table 1a). Dot-blot hybridizations using RNA extracted from one of these lines showed that the levels of endogenous $\alpha 2(I)$ collagen RNA and those of *cat* RNA decreased 8-10-fold after MMSV transformation, whereas the levels of actin RNA were unchanged (Fig. 3b).

The plasmid construction used in these experiments also harbours the early promoter of SV40, which is fused to the bacterial *gpt* gene (Fig. 1a); this SV40 promoter segment includes the SV40 enhancer sequences^{14,15}. To determine whether SV40 enhancer sequences are required for inhibition of the $\alpha 2(I)$ collagen promoter after MMSV transformation, NIH 3T3 cells were transfected with pAZ1005, a plasmid in which a mouse $\alpha 2(I)$ collagen promoter segment is fused to the *neo* gene but which does not contain SV40 enhancer sequences (Fig. 1c). Several colonies resistant to the antibiotic G418 (positive for the expression of the *neo* gene) were isolated and transformed either by MMSV or by a plasmid containing the



As a control for these experiments, we used cell lines that had been transfected with either one of two plasmids containing the chicken β -actin promoter. The first plasmid (p $\beta 6$ actin) contains the entire chick β -actin gene cloned in PUC-8 (a gift of B. Paterson). NIH 3T3 cells were co-transfected with p $\beta 6$ actin and pSV2-neo, and G418-resistant colonies were selected. Figure 4 shows that the levels of β -actin RNA correctly initiated from the transfected β -actin promoter are not reduced after MMSV transformation, but in the same cells, the levels of endogenous $\alpha 2(I)$ collagen RNA are sharply decreased (data

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not shown). The other plasmid used (pAZ1037) contains a fragment of the chicken β -actin gene, including 350 bp preceding the start of transcription, the first exon, the first intron and part of the second exon fused to the *cat* gene. The plasmid is otherwise identical to pAZ1009 and, therefore, contains the same splice signals and polyadenylation site, in addition to the SV40 enhancer segment. NIH 3T3 cells were co-transfected with

this plasmid and pSV2-neo, G418-resistant colonies were selected and the levels of CAT compared before and after transformation of the colonies with MMSV. The results (Table 1c) indicate that the levels of CAT show little change in most colonies after transformation by MMSV. When a decrease occurs, it is clearly less than in colonies containing a transfected $\alpha 2(I)$ collagen promoter. This experiment suggests that neither the *cat* DNA sequences nor the sequences spanning the SV40 splice signals, the intron and the poly(A) addition site are responsible for the reduced levels of *cat* RNA found in the MMSV-transformed cells in which these DNA elements are fused to the $\alpha 2(I)$ collagen promoter. Hence, the decrease in *cat* RNA levels in such cells is presumably not caused by a change in the stability of these RNA sequences.

The AT3-14 cell line is an NIH 3T3 cell that has been stably transfected with plasmid pHO1000. In these cells, the *gpt* gene is under the control of the early SV40 promoter, whereas the *cat* gene is transcribed from the $\alpha 2(I)$ collagen promoter (Figs 1, 2). Comparison of the levels of *gpt* RNA in these cells before and after transformation by MMSV revealed that the levels of *gpt* RNA were also strongly decreased after MMSV transformation (Fig. 3d, lanes 1, 2). One possible explanation for the down-regulation of the early SV40 promoter in MMSV-transformed cells is a *cis* effect mediated by the $\alpha 2(I)$ collagen promoter present on the same plasmid. NIH 3T3 cells were, therefore, co-transfected with pSV2-*cat* and pSV2-neo, and G418-resistant colonies were isolated and transformed by

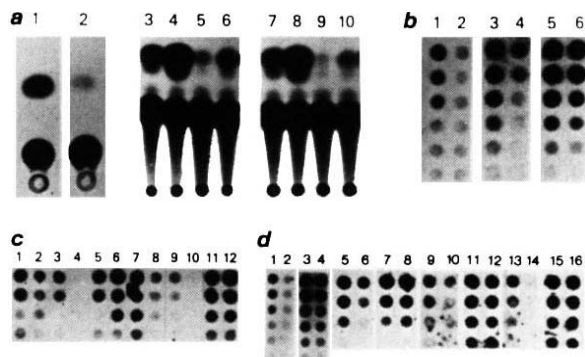


Fig. 3 Expression of the recombinant $\alpha 2(I)$ collagen promoter (a-c) and of the recombinant SV40 early promoter (d) in normal and transformed cells. Clones of NIH 3T3 stably transfected with pHO1000 (a, b) or pAZ1005 (c) were withdrawn from the selection medium and transformed by a mixture of murine Moloney sarcoma virus and murine leukaemia virus (MMSV) or by transfection with the *v-mos* gene. a, Levels of CAT activity in normal and MMSV-transformed clones of NIH 3T3 cells stably transfected with pHO1000. 5×10^5 cells, normal (lanes 1, 3, 4, 7, 8) and transformed (lanes 2, 5, 6, 9, 10), were seeded in 100-mm dishes. After 48 h, the level of CAT was measured as described by Gorman *et al.*¹², except that equal amounts of proteins were used in the assays: lanes 1 and 2, clone AT3-14, 0.4 mg protein extract per assay; lanes 3-6, clone AT1; lanes 7-10, clone AT2; lanes 3, 5, 7, 9, 0.15 mg protein per assay; lanes 4, 6, 8, 10, 0.75 mg protein per assay. b, RNA dot blots from clone AT3-14. 1.5×10^6 AT3-14 cells (lanes 1, 3, 5) or MMSV-transformed AT3-14 cells (lanes 2, 4, 6) were seeded in 100-mm dishes. After 24 h, total RNA was extracted and twofold serial dilutions (in $20 \times$ SSC with $25 \mu\text{g ml}^{-1}$ bacterial rRNA) of total RNA (starting with $10 \mu\text{g}$ RNA per dot) were applied to nitrocellulose paper using a dot-blot template. The levels of $\alpha 2(I)$ collagen RNA (lanes 1, 2), *cat* RNA (lanes 3, 4) and actin RNA (lanes 5, 6) were measured using the hybridization probes described in Fig. 2 legend. c, RNA dot-blot hybridization of clones of NIH 3T3 stably transfected with the plasmid pAZ1005. RNA was isolated and spotted for dot-blot hybridization as described in b. Lanes 1-6, clone AZ51; lanes 7-12, clone AZ57; lanes 1, 3, 5, 7, 9, 11 are of RNA from normal cells; lanes 2, 4, 6, 8, 10 and 12 are of RNA from *v-mos*-transformed cells; lanes 1, 2, 7 and 8 were hybridized with the same $\alpha 2(I)$ collagen probe. Lanes 3, 4, 9 and 10 were hybridized with a *neo* probe consisting of a 1,300-bp *Sma*I/*Hind*III fragment of pAZ1005; lanes 5, 6, 11 and 12 were hybridized with the β -actin probe. d, Activity of the early SV40 promoter in normal and MMSV-transformed NIH 3T3 cells. Cells were stably transfected with plasmids pHO1000 (lanes 1-4, clone AT3-14) or pSV2-neo (lanes 5-8, clone SV7; lanes 9-12, clone SV8; lanes 13-16, clone SV9). RNA was extracted from normal cells (lanes 1, 3, 5, 7, 9, 11, 13, 15) or MMSV-transformed cells (lanes 2, 4, 6, 8, 12, 14) and twofold serial dilutions of the RNA were spotted and hybridized as described in b. Lanes 1 and 2 were hybridized with a *gpt* DNA probe; lanes 5, 6, 9, 10, 13 and 14 were hybridized with a *neo* DNA probe; lanes 3, 4, 7, 8, 11, 12, 15 and 16 were hybridized with a β -actin DNA probe.

Methods. Transformation by MMSV: Cells were seeded in two T-25 flasks (3×10^5 cells per flask); to one of the flasks 5 ml of medium, incubated for 3 h with established MMSV-transformed NIH 3T3 cells, were added. Both normal and infected cells were carried for 1-2 weeks before they were assayed. Transformation by *v-mos*: clones of cells stably transfected with pAZ1005 and expressing the *neo* gene were isolated as described in Fig. 2 legend with the exception that *neo* selection medium was used ($600 \mu\text{g ml}^{-1}$ G418). G418-resistant clones were re-transfected by plasmid pHT25, carrying the *v-mos* gene²⁴ (gift of G. Van de Woude). The transformed colonies were selected by focus formation and recloned in soft agar.

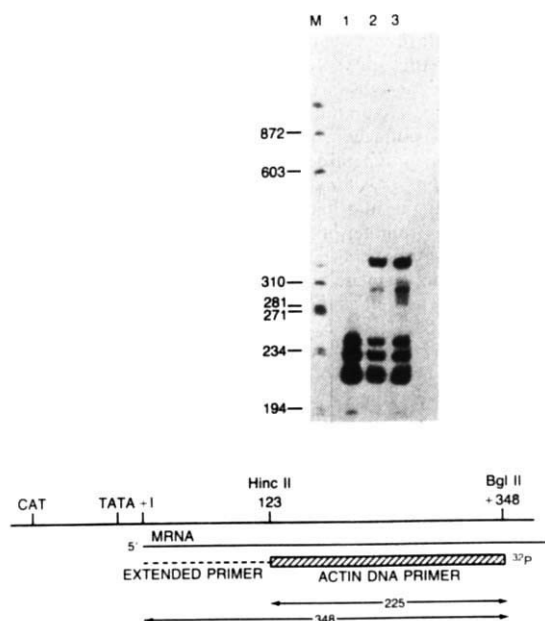


Fig. 4 Levels of chick β -actin RNA in normal and MMSV-transformed NIH 3T3 cells stably transfected by the chicken β -actin gene (p $\beta 6$ actin)¹⁹. A genomic DNA (18 μg) fragment containing the entire chicken β -actin gene cloned in PUC-8 (a gift of B. Paterson) was co-transfected with 2 μg DNA of pSV2-neo¹³ into NIH 3T3 cells. G418-resistant clones (~ 100 colonies) were pooled and transformed by MMSV as described in Fig. 3 legend. Total RNA was extracted by the guanidine hydrochloride method²¹ and the levels of chick β -actin RNA were measured using a primer extension method. A single-strand 225-nucleotide *Bgl*II/*Hinc*II fragment of the chicken β -actin cDNA clone, labelled with ^{32}P at its *Bgl*II 5' end, was used as primer and hybridized to total RNA of untransfected NIH 3T3 cells (lane 1), to untransformed NIH 3T3 cells stably transfected with pAZ1037 (lane 2) and to the same cell transformed with MMSV (lane 3). The primer was extended with reverse transcriptase as described for Fig. 2 and the products were analysed by electrophoresis on a 7 M urea/5% polyacrylamide gel. The bands on the gel were quantitated by microdensitometry; the ratio of the 348-nucleotide cDNA to the 225-nucleotide primer was 0.55 in lane 2 and 0.62 in lane 3.

Table 1 Levels of activity of transfected $\alpha 2(I)$ collagen, β -actin and SV40 early promoter in normal and MMSV-transformed cells

Cell lines	Promoter	CAT specific activity (c.p.m. per mg protein $\times 10^4$)		Ratio (N/T)
		Normal (N)	Transformed (T)	
a 1, AT3-14 } 2, AT1 } 3, AT2 }	Chick $\alpha 2$ type I collagen	19.5	2.8	7.0
		32.0	2.2	14.5
		18.7	1.3	14.4
b 1, AZC1 } 2, AZC2 } 3, AZC4 } 4, AZC5 } 5, AZC6 }	Mouse $\alpha 2$ type I collagen	25.8	5.4	4.8
		236.9	60.7	3.9
		24.1	3.9	6.2
		86.1	3.9	22.1
		641.5	504.7	1.3
c 1, AZA1 } 2, AZA2 } 3, AZA3 } 4, AZA4 } 5, AZA6 }	Chick β -actin	522.1	362.7	1.4
		118.5	70.6	1.6
		3.3	2.7	1.2
		35.4	11.7	3.0
		41.0	20.3	2.0
d 1, SV1 } 2, SV5 } 3, SV6 }	SV40 early region	214.2	34.7	6.2
		139.3	10.8	12.9
		0.5	0.2	2.5

NIH 3T3 cells were transfected with 10 μ g pHO1000 DNA (as described in Fig. 1 legend) (a, 1-3) or with 10 μ g DNA of pSV2-neo and pAZ1009 (b, 1-5), pSV2-neo and pAZ1037 (c, 1-5) or pSV2-neo and pSV2-cat (d, 1-3). The ratio of pSV2-neo DNA to the other DNA used in co-transfections was 1:10. Individual colonies resistant to mycophenolic acid (a) or to G418 (b, c, d) were isolated. Levels of CAT were measured before and after MMSV transformation of these cells as described in Fig. 2 legend.

MMSV. Table 1d compares the levels of CAT in these cells before and after MMSV transformation and shows clearly that the enzyme levels are strongly reduced on transformation. In a similar experiment, NIH 3T3 cells were transfected with pSV2-neo alone, and G418-resistant colonies were isolated and transformed by MMSV. Comparison of the levels of *neo* RNA in these cells before and after transformation by MMSV showed that in almost all cell lines the levels of *neo* RNA were decreased after transformation (Fig. 3d, lanes 5, 6; 9, 10; 13, 14). These experiments suggest that the activity of the early promoter of SV40 is also inhibited in MMSV-transformed cells and that this effect is not mediated in *cis* via the $\alpha 2(I)$ collagen promoter. Because a wide range of changes occurs in the pattern of proteins synthesized in transformed cells, it is not unexpected that another promoter would also be inhibited in transformed fibroblasts. We speculate that the SV40 early promoter contains sequence elements that might interact with one or more proteins which also recognize the $\alpha 2(I)$ collagen promoter in fibroblasts. One possible hypothesis is that one or more of these proteins may either be modified or may cease to be synthesized after MMSV transformation.

The decrease in *cat* or *neo* RNA levels in cells where the corresponding genes are linked to the $\alpha 2(I)$ collagen promoter is a result of the presence in these cells of an active transforming protein. Indeed, when AT3-14 cells, a cell line in which the $\alpha 2(I)$ collagen promoter is fused to the *cat* gene, were transformed with a mutant of the Kirsten murine sarcoma virus, temperature-sensitive for transformation, the levels of CAT activity were decreased at the permissive temperature but not at the non-permissive temperature. These changes parallel the changes in the levels of endogenous $\alpha 2(I)$ collagen RNA (data not shown).

Thus, the activity of the $\alpha 2(I)$ collagen promoter introduced into NIH 3T3 cells by DNA transfection is strongly inhibited after malignant transformation by the *v-mos* oncogene. This inhibition parallels the reduction in the expression of both the endogenous type (I) collagen and fibronectin¹⁶ genes in fibroblasts. A similar decrease is seen in the activity of the transfected SV40 early promoter, but not in the activity of a transfected β -actin promoter.

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- Unkeless, J. C. *et al.* *J. exp. Med.* **137**, 85-112 (1978).
- Dulak, N. C. & Temin, H. M. *J. cell. Physiol.* **81**, 153-160 (1973).
- Gottesman, M. M. & Sobel, M. E. *Cell* **19**, 449-455 (1980).
- Green, H., Todaro, G. J. & Goldberg, B. *Nature* **209**, 916-917 (1966).
- Levinson, W., Bhatnagar, R. S. & Liu, T. *J. natn. Cancer Inst.* **55**, 807-810 (1975).
- Hata, R. & Peterkofsky, B. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2933-2937 (1977).
- Howard, B. H., Adams, S. L., Sobel, M. E., Pastan, I. & de Crombrughe, B. *J. biol. Chem.* **253**, 5869-5974 (1978).
- Sobel, M. E., Yamamoto, T., de Crombrughe, B. & Pastan, I. *Biochemistry* **20**, 2678-2684 (1981).
- Avvedimento, E. *et al.* *Nucleic Acids Res.* **9**, 1123-1131 (1981).
- Sandmeyer, S., Gallis, B. & Bornstein, P. *J. biol. Chem.* **256**, 5022-5028 (1981).
- Ohkubo, H. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **77**, 7059-7063 (1980).
- Gorman, C. M., Moffat, L. & Howard, B. H. *Molec. cell. Biol.* **2**, 1044-1051 (1982).
- Southern, P. J. & Berg, P. *J. molec. appl. Genet.* **1**, 327-341 (1982).
- Banerji, J., Rusconi, S. & Schaffner, W. *Cell* **27**, 299-308 (1981).
- Wasylyk, B., Wasylyk, C., Auger, P. & Chambon, P. *Cell* **32**, 503-514 (1983).
- Vogel, G. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **78**, 5334-5338 (1981).
- Mulligan, R. & Berg, P. *Science* **209**, 1422-1427 (1980).
- Schmidt, A., Yamada, Y. & De Crombrughe, B. *J. biol. Chem.* **259**, 7411-7415 (1984).
- Seiler-Tuyns, A., Eldridge, J. D. & Paterson, B. M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2980-2984 (1984).
- Goldfarb, D. S., Rodriguez, R. L. & Doi, R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5886-5890 (1982).
- Sobel, M. E. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **75**, 5846-5850 (1978).
- Goldberg, D. A. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5794-5798 (1980).
- Cleveland, D. W. *et al.* *Cell* **20**, 95-105 (1980).
- Blair, D. G. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **77**, 3504-3508 (1980).

High-affinity binding site for a specific nuclear protein in the human IgM gene

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Proteins binding to specific regions of DNA with high affinity frequently govern or regulate reactions at the gene level¹⁻⁸. We have identified a high-affinity binding site in the immunoglobulin μ gene that binds a specific nuclear protein, and have now characterized it fully using nuclear factor 1 (NF-1), a protein purified from the nuclei of HeLa cells^{9,10} and required for the *in vitro* replication of adenovirus (Ad) DNA⁹⁻¹². NF-1 protects a 25-base pair (bp) double-stranded segment of DNA which shares a consensus sequence, 5' TGGA/CNNNNNGCCAA 3', with similar

binding sites in the Ad-5 terminal repeat¹⁰⁻¹² and the human *c-myc* gene¹³. Although this site differs from the enhancer region¹³⁻¹⁶, a biological function is suggested by the fact that it is DNase I hypersensitive in immunoglobulin-producing lymphoblastoid cells. The binding site for the NF-1 protein in the μ gene, by analogy with the site in the Ad-5 terminal repeat, may represent one component of a cellular origin of replication; alternatively, it may be responsible for the activation of the chromatin in this region.

The immunoglobulin heavy-chain genes represent a set of DNA sequences of intense transcriptional activity in

immunoglobulin-producing B cells. They also represent a target for a highly specific translocation event that delivers the oncogene *c-myc* into this locus in most instances of the B-cell malignancy, Burkitt's lymphoma^{17,18}. Recently, we have identified five potential regulatory sites in the *myc* gene region on the basis of their DNase I hypersensitivity¹³. Two of these sites,

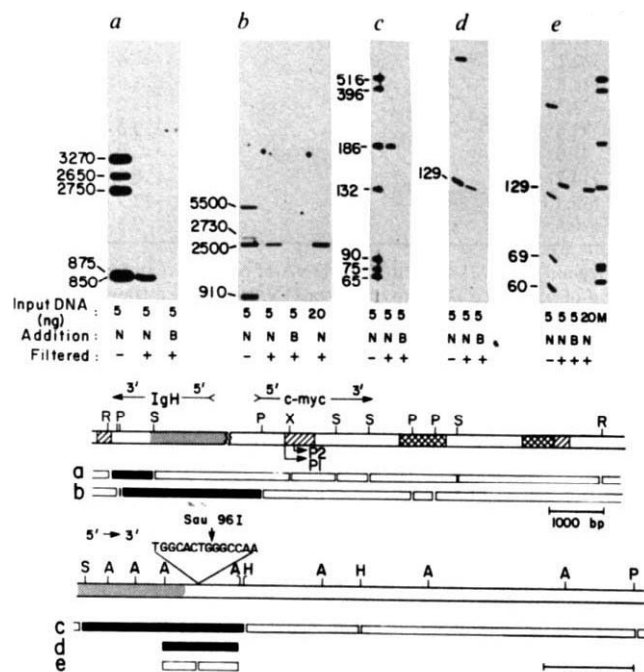


Fig. 1 Mapping of the protein binding sites on DNA fragments overlapping the human IgM and *myc* genes. Recombinant plasmids were cut with restriction enzymes and the resulting fragments were end-labelled using [α^{32} P]-dNTPs (labelled deoxynucleotide triphosphates) and the large fragment of *Escherichia coli* polymerase (Klenow)¹³. Labelled DNA was incubated with a low salt extract from Burkitt's lymphoma (BL22) nuclei (N)¹³ for 30 min at 25 °C and passed through nitrocellulose filters. Protein-bound DNA, retained on the filters, was extracted¹³ and analysed on agarose gels (a, b) (+) or sequencing gels (c-e) (+). The pattern of input DNA was assayed by omitting the nitrocellulose filtration step (-). Background binding of labelled fragments on nitrocellulose filters was analysed by substituting bovine serum albumin (B) for nuclear proteins. To determine protein binding sites, cloned DNA from BL22, overlapping the IgM gene and *myc* gene (pBL22 RI-RI 9.0)¹⁶, was cut with *Eco*RI, *Sst*I and *Xho*I (a) or with *Pst*I (b). a, *Eco*RI and *Xho*I sites; b, *Pst*I sites were labelled radioactively. For fine mapping, the 600-bp *Sst*I/*Pst*I fragment (sequence is shown in Fig. 2c) was cloned into the polylinker of the vector pUC13 (BRL) (pIgHSP0.6) and cut with *Eco*RI and *Hinf*I (c). Both types of ends were labelled radioactively. The *Eco*RI site is in the polylinker next to the *Sst*I site. The *Alu*I fragment covering the protein binding site was cloned into the *Sma*I site of M13mp10 RF (M13IgHA80). d, e, Binding assays where this fragment was cut out with *Eco*RI and *Hinf*I, the sites which bracket the polylinker. e, M13IgHA80 cut with *Eco*RI and *Hinf*I was recut with *Sau*96I. The 129-bp *Eco*RI/*Hinf*I fragment is cut into two 60 and 69-bp fragments. The other two fragments are derived from M13mp10 RF. As a positive control for binding, 20 ng M13IgHA80 cut with *Eco*RI and *Hinf*I were added to the samples to be filtered. Solid bars, DNA fragments bound by nuclear proteins; dotted regions, μ switch region; single-hatched regions indicate non-translated exon sequences. Double hatched regions indicate protein coding sequences. P1 and P2, the two promoters of the *myc* gene. A, *Alu*I; R, *Eco*RI; H, *Hinf*I; P, *Pst*I; S, *Sst*I; X, *Xho*I. As a relative molecular mass marker (M) in e, the plasmid pIgHSP0.6 had been digested with *Hinf*I and *Eco*RI. The visible bands indicate the following sizes: 516, 396, 186, 132, 84, 78 and 68 bp.

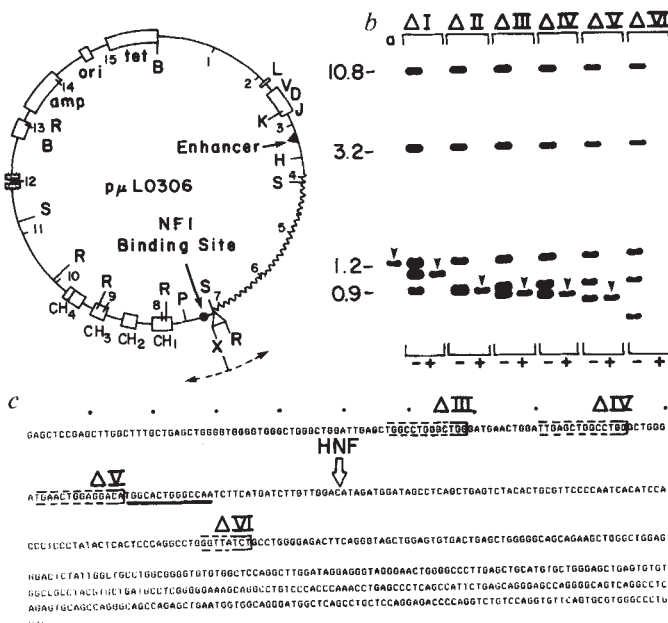


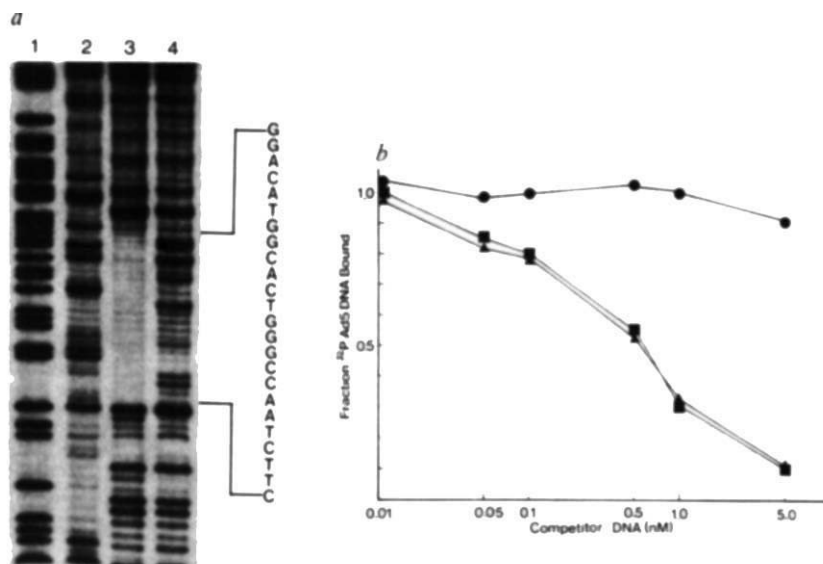
Fig. 2 Fine mapping of the NF-1 binding site in an IgM gene. a, Construction of p μ L0306: B, *Bam*HI; K, *Kpn*I; P, *Pst*I; R, *Eco*RI; S, *Sst*I. Wavy line, μ switch region; open boxes drawn with wavy lines, IgM membrane exons, the exact location of which is not known; open boxes with straight lines represent IgM exons. b, Mapping the protein binding sites on the IgM plasmids carrying the *Bal*31 deletions; the sizes of the *Eco*RI fragments not subjected to *Bal*31 deletions are always indicated by an arrow. The numbers above the upper brackets indicate the different plasmids used. Below the lower brackets, lanes with non-filtered (-) and filter-bound (+) DNA are indicated. c, DNA sequence of the 603-bp *Sst*I/*Pst*I fragment. The DNA sequence was derived by using the dideoxy chain terminating method according to the BRL Manual (BRL, Inc.). The brackets indicate the endpoints of the various deletions. Sequences to the left of the brackets were deleted and replaced with *lacZ* DNA. The numbers above the brackets indicate the respective plasmid. The underlined sequence is the core of the proposed NF-1 binding site (see Fig. 3a). The arrow above the sequence indicates the midpoint of the DNase I hypersensitive region (HNF) (see Fig. 4).

Methods. a, A pBR322 molecule was linearized by *Bam*HI and, in a series of steps, ligated to three pieces of DNA from human μ libraries to produce a cloned rearranged human μ plasmid. The region from the *Bam*HI site at ~12.8 kb to the *Hind*III site at ~3.7 kb came from a germline library, the region from this *Hind*III site to the *Kpn*I site at ~2.7 kb from a lymphoma library (line LR35) and the region from this *Kpn*I site to the *Bam*HI site at 0 kb from a closely related lymphoma library (line LR36). Into the germline piece was inserted (as shown) a 268-bp *Sst*I/*Sst*I fragment containing M13mp11 sequences from the *Sst*I site in the polylinker to the *Pvu*III site in the *lac*I gene (the latter converted to an *Eco*RI site with an *Eco*RI linker), joined to pBR327 sequences from the *Eco*RI site to the *Cl*aI site (the latter converted to an *Sst*I site by blunting the ends with Klenow polymerase and adding an *Sst*I linker). The unique *Xho*I site had been produced by cloning an *Xho*I linker into the M13mp11 *Sma*I site. Not all restriction sites are shown for all enzymes. Following *Bal*31 deletion starting at the unique *Xho*I site, the plasmids were re-ligated after blunting the ends with Klenow polymerase. b, Six plasmids carrying different deletions were cut with *Eco*RI labelled by fill-in and incubated with nuclear extract. Non-filtered (-) as well as filter-bound (+) DNA was subjected to agarose gel electrophoresis (for details see Fig. 1).

Fig. 3 *a*, DNase I footprint of the NF-1 binding site in the IgM gene. *b*, Competition nitrocellulose filter-binding assays with Ad-5 and IgM NF-1 binding sequences.

Methods. *a*, A 32 P-end-labelled DNA fragment from M13IgHA80 RF DNA containing a NF-1 binding site was subjected to limited DNase I digestion in the absence (lane 3) or presence (lane 4) of the phosphocellulose fraction¹⁰ of NF-1 protein and the products were analysed on an 8% polyacrylamide urea sequencing gel. G+A (lane 1) and A+C (lane 2) sequencing reactions of the fragment were run in parallel lanes to determine the sequence of the region protected by the protein. M13IgHA80 RF DNA was linearized by restriction with *Eco*RI and the 3' recessed ends of the fragments were filled-in and labelled using DNA polymerase I large fragment (Klenow) and [α - 32 P]dATP (3,000 ci mmol⁻¹). The labelled DNA fragments were cut with *Hind*III and the fragment containing the NF-1 binding site isolated by electrophoresis in a 2% agarose gel and extracted by electroelution followed by chromatography through NACs resin (BRL). Protein-DNA

binding was carried out at 0 °C for 20 min in a reaction mixture (40 μ l) containing 50 mM HEPES (pH 7.5), 5 mM MgCl₂, 1 mM dithiothreitol DTT, 250 μ g ml⁻¹ bovine serum albumin, 125 μ g ml⁻¹ *E. coli* DNA, 200 mM NaCl, 15 μ l phosphocellulose I fraction of NF-1 and 1 ng 32 P-DNA fragment. The reaction mixtures were incubated with DNase I (0.075 μ g) for 1 min at 23 °C, extracted with neutralized phenol and, after ethanol precipitation, the reaction products analysed as described above. *b*, The binding affinities of NF-1 to Ad-5 and IgM DNA were compared by competition nitrocellulose filter-binding assays as described previously¹⁰. Purified NF-1 (200 ng) and 5 fmol *Eco*RI-restricted 32 P-labelled pMDC10 DNA (contains 365 bp of Ad-5 left-terminal sequence) were incubated with varying amounts of unlabelled form I pMDC10 and pIgHSP0.6. The concentration of labelled pMDC10 *Eco*RI bound to NF-1 was determined by nitrocellulose filter binding. The fraction bound was calculated as the ratio of Ad-5 DNA bound to the filter in the presence and absence of competitor DNA. The following DNAs were used as competitors: ●, pUC9 (plasmid DNA); ■, pMDC10 (Ad-5/NF-1 binding site); ▲, pIgHSP0.6 (IgM/NF-1 binding site).



a putative repressor binding site and a weak NF-1 binding site, are not associated with the translocated and deregulated *myc* gene in the Burkitt's lymphoma cell line, BL22 (refs 19, 20). Therefore *myc* activation could be the result of *cis*-acting sequences in the immunoglobulin heavy-chain locus (*Igh*). To identify such DNA sequences we sought to find regions in the translocated immunoglobulin μ segment which might interact specifically with nuclear proteins. Using a nitrocellulose filter binding assay^{13,21,22} and cloned DNA (pBL22 RI-RI 9.0; see Fig. 1), including the IgM/*myc* crossover region in BL22 (ref. 16). We discovered a fragment of DNA between the μ switch and the μ constant region binding specifically to protein(s) derived from a low-salt BL22 nuclear extract¹³. The binding region is in a 603-bp DNA segment between a *Pst*I and *Sst*I site (see Fig. 1a, b). Subcloned restriction fragments of this 603-bp fragment were assayed and the binding site further mapped to a 80-bp *Alu*I fragment (Fig. 1c, d). Recutting a 129-bp fragment containing the 80-bp *Alu*I fragment with *Sau*96I destroyed the ability of the resulting fragments (69 and 60 bp, see Fig. 1e) to be retained in the filter binding assay, suggesting that the binding site either contains the *Sau*96I site or lies very close to it. The binding site is also present in the germline configuration of the *Igh* locus (Fig. 2), indicating that mutational events in BL22 are not responsible for its appearance. Further information about the location and nature of the protein binding site was obtained using *Bal*31 deletions, sequence analysis and DNase I footprinting *in vitro* (Figs 2, 3). Using a normal μ gene, we constructed deletions starting from a unique *Xho*I site cloned into the *Sst*I site at the 3' end of the μ switch (see Fig. 2a) and extending up to and through the putative binding site (Fig. 2). Whereas deletions Δ I- Δ V do not destroy the binding site, deletion Δ VI, which ends 3' of the normally bound *Alu*I fragment (Fig. 1d), abolishes binding (Fig. 2b).

The sequence of this region suggested that the bound nuclear protein might be NF-1. This protein binds to two homologous sequences in Ad-5 (refs 10, 13) and the *c-myc* gene¹³. Here we show that the homology extends to a sequence in the μ -gene fragment which is recognized in the filter binding assay (Figs 1d, 2c). To confirm this, the pure NF-1-DNA interaction was visualized by DNase I footprinting analysis (Fig. 3a). Although

NF-1 protects ~25 bp, sequence comparisons suggest that only a core sequence in the centre of the protected area is necessary for the specific protein DNA interaction (Fig. 2). By comparing the sequence in the IgM gene protected by NF-1 (Fig. 3a) with the published NF-1 sites in Ad-5 (refs 10, 12) and the *myc* gene¹³ and unpublished sites in other adenovirus serotypes as well as in the human polyomavirus JC and the human and simian cytomegalovirus (D.R., P.R., G. Hayward, P. Padmanabhan and L.H., unpublished results), a consensus sequence can be derived (5'... TGGA/ C N N N N G C C A A ... 3'). In addition, replacement of the sequences upstream of the TGG... (Fig. 2c) with *lacZ* sequences (data not shown) in deletion mutant Δ V does not interfere with binding of NF-1 (Fig. 2b). A DNA binding protein from chicken oviduct nuclei recognizes a similar sequence in the chicken lysozyme gene region²³.

The binding site exhibits a 2-fold axis of symmetry exemplified by the TGGC... GCCA sequences. High binding affinity occurs when this symmetry is most pronounced, as in the IgM and Ad-5 binding sites^{10,13}. Using an equilibrium competition assay, we show that the IgM and Ad-5 binding site affinities are nearly equal (Fig. 3b) and an order of magnitude stronger than the *c-myc* binding site, which exhibits only an approximate symmetry¹³. The 2-fold symmetry axis in the binding site suggests that NF-1 binds as a dimeric form mechanistically similar to repressor protein DNA interactions²⁴. Although it is not known whether NF-1 purified from HeLa cells and the binding protein(s) from B cells are identical, we have shown that both binding activities recognize the same synthetic binding site cloned into a plasmid vector (L.H. and L. Davis, in preparation).

To assign biological significance to the NF-1 binding site in the IgM gene, we provide evidence that NF-1 also binds to this element *in vivo*. The localization of DNase I hypersensitive sites in chromatin can identify potential protein binding sites^{3,25,26}. Therefore, we mapped the DNase I hypersensitive sites in the IgM gene in lymphoblastoid cells and in BL22 (Fig. 4). A probe from the μ constant region detects two DNase I hypersensitive sites in the lymphoblastoid cell line 8392 (Fig. 4A, a). One in the μ switch, approximately where the *myc* μ translocation point in BL22 is found, and one near the NF-1 binding site. NF-1 binds ~3.1 kilobases (kb) 5' to the *Xba*I site (Fig. 4B, a) and

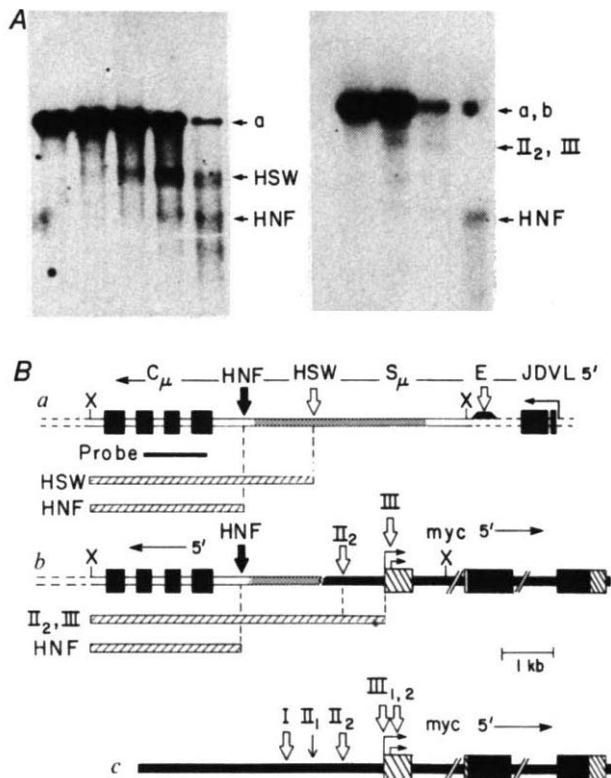


Fig. 4 *A*, Mapping of DNase I hypersensitive sites near the *c-myc* gene and IgM gene in the lymphoblastoid cell line 8392 (left) and the Burkitt's lymphoma cell line BL22 (right). Nuclei of both cells were digested with increasing amounts of DNase I as described previously¹³ and then 15 μ g of isolated genomic DNA restricted with *Xba*I, electrophoresed on a 0.8% agarose gel, blotted onto nitrocellulose and hybridized. A 1.2-kb *Eco*RI fragment overlapping the μ constant region was used as hybridization probe. *B*, The probe hybridizes to a germline IgM fragment (*a*) and to a fragment of similar size from the IgM *myc* translocation product (*b*). The DNase I hypersensitive sites are labelled HSW (hypersensitive switch), HNF (hypersensitive NF-1 binding site), E (enhancer)¹³⁻¹⁶, I, II1, II2, III1, and III2 as described previously¹³ and their positions are indicated on the map (X, *Xba*I). *a*, *b*, Solid arrow indicates the binding site for NF-1; cross-hatched bars, sub-bands in the cell lines 8392 (*a*) and BL22 (*b*). Protein coding regions of both the *myc* gene and the IgM gene are indicated by solid boxes, nontranslated regions of exons by cross-hatched boxes. Dotted regions correspond to the μ switch. The *myc* gene is not drawn to scale. *c*, Pattern of DNase I hypersensitive sites in the germline *myc* is shown¹³.

therefore falls into the DNase I hypersensitive region represented by a sub-band 3,050–3,200-bp long (Fig. 4*B*, *a*). The same probe picks up two sub-bands in BL22 (Fig. 4*B*, *b*), one representing the DNase I hypersensitive region at the NF-1 binding site and one representing closely linked sites in the *myc* gene translocated into the IgM region. This band has a size of ~5 kb and presumably corresponds to the sites II2, III1 and III2 (Fig. 4*B*, *b*, *c*) occurring in the 5' portion of the *myc* gene¹³. The co-localization of DNase I hypersensitive sites and NF-1 binding sites in the *myc*¹³ and IgM genes suggests a possible NF-1 DNA interaction *in vivo*.

The function of the μ NF-1 binding site remains to be defined. It could be similar to that of the analogous sequence in adenovirus, that is, acting as one component of an origin of replication. In the HeLa cell genome, the frequency of high-affinity NF-1 binding sites is one per 100 kb²⁷, a spacing similar to that of cellular origins of replication²⁸. Alternatively, these sites could be involved in transcriptional activation, although this is not consistent with the distribution of NF-1 binding sites in the immunoglobulin heavy-chain locus. The NF1 site is in the first intron of the μ gene just 3' to the μ -switch region. A search in clones overlapping the γ and ϵ switch and constant regions failed to reveal any binding sites (data not shown). During B-cell development, a series of recombination events leads to a switch in heavy-chain constant-region class from μ/δ to either γ , ϵ or α ^{29,30}. After switching from μ/δ to any other constant region, the μ NF-1 binding site would be deleted from the resulting active heavy-chain gene. By contrast, the canonical *Igh* enhancer 5' to the μ switch is retained during heavy-chain class switching¹⁴⁻¹⁶. This, together with the fact that equivalent binding sites do not occur at corresponding positions in the γ and ϵ genes, makes it less probable that NF-1 acts as a simple transcriptional enhancer. On the other hand, the loss of the μ NF-1 binding site during heavy-chain class switching could indicate that, in the *Igh* region, NF-1 is a necessary component for this 'dead end' recombination process. We are left with another, equally attractive, possibility, that the NF-1 binding sites have a more universal role in the activation of chromatin during early stages of cell differentiation, suggested by the observation that the NF-1 binding sites in the human *myc*¹³ and IgM genes are located in DNase I hypersensitive regions, areas associated with the active state of chromatin.

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1. Gilbert, W., Maizels, N. & Maxam, A. *Cold Spring Harb. Symp. quant. Biol.* **38**, 845–855 (1974).
2. Maniatis, T. *et al. Cell* **5**, 109–113 (1975).
3. Dynan, W. S. & Tjian, R. *Cell* **35**, 79–87 (1983).
4. Jones, K. A. & Tjian, R. *Cell* **36**, 155–162 (1984).
5. Compton, J. G., Schrader, W. T. & O'Malley, B. W. *Proc. nat. Acad. Sci. U.S.A.* **80**, 16–20 (1983).
6. Karin, M. *et al. Nature* **308**, 513–519 (1984).
7. Renkawitz, R., Schutz, G., von der Ahe, D. & Beato, M. *Cell* **37**, 503–510 (1984).
8. Chandler, V. L., Maler, B. A. & Yamamoto, K. R. *Cell* **33**, 489–499 (1983).
9. Nagata, K., Guggenheimer, R. A., Enomoto, T., Lichy, J. H. & Hurwitz, J. *Proc. nat. Acad. Sci. U.S.A.* **79**, 6438–6442 (1982).
10. Rawlins, D. R., Rosenfeld, P. J., Wides, R. J., Chailberg, M. D. & Kelly, T. J. Jr *Cell* **37**, 309–319 (1984).
11. Nagata, K., Guggenheimer, R. A. & Hurwitz, J. *Proc. nat. Acad. Sci. U.S.A.* **80**, 6177–6181 (1983).
12. Guggenheimer, R. A., Stillman, B. W., Nagata, K., Tamanoi, F. & Hurwitz, J. *Proc. nat. Acad. Sci. U.S.A.* **81**, 3069–3073 (1984).
13. Siebenlist, U., Hennighausen, L., Battey, J. & Leder, P. *Cell* **37**, 381–391 (1984).
14. Hayday, A. *et al. Nature* **307**, 334–340 (1984).
15. Rabbitts, T. H., Hamlyn, P. H. & Baer, R. *Nature* **306**, 760–765 (1983).
16. Bannerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729–740 (1983).
17. Taub, R. *et al. Proc. nat. Acad. Sci. U.S.A.* **79**, 7837–7841 (1982).
18. Leder, P. *et al. Science* **222**, 765–771 (1983).
19. Battey, J. *et al. Cell* **34**, 779–789 (1983).
20. Taub, R. *et al. Cell* **36**, 339–348 (1984).
21. Riggs, A. D., Bourgeois, S. & Cohn, M. *J. molec. Biol.* **53**, 401–417 (1970).
22. Jack, R. S., Gehring, W. J. & Brack, C. *Cell* **24**, 321–333 (1981).
23. Borgmeyer, U., Nowock, J. & Sippel, A. E. *Nucleic Acids Res.* **12**, 4295–4311 (1984).
24. Pabo, C. & Lewis, A. *Nature* **298**, 443–447 (1982).
25. Shermoen, A. W. & Beckendorf, S. K. *Cell* **29**, 601–607 (1982).
26. Emerson, B. M. & Felsenfeld, G. *Proc. nat. Acad. Sci. U.S.A.* **81**, 95–99 (1984).
27. Gronostajski, R. M., Nagata, K. & Hurwitz, J. *Proc. nat. Acad. Sci. U.S.A.* **81**, 4013–4017 (1984).
28. Lewin, B. M. *Gene Expression* 2nd edn (Wiley, New York, 1980).
29. Leder, P. *Sci. Am.* **246**(5), 102–115 (1982).
30. Shimizu, A. & Honjo, T. *Cell* **36**, 801–803 (1984).

Eastern and Western approaches

Roy MacLeod

The Touch of Midas: Science, Values and Environment in Islam and the West.

Edited by Ziauddin Sardar.

Manchester University Press/Longwood University Press: 1984. Pp.253. £20, \$35.

VISITORS to the Science Museum in London during the spring and summer of 1976 were greeted by an unusual exhibition. Conceived by Professor S.H. Nasr, then Director of the Iranian Academy of Science, there was on view a rich display of science and technology in Islam; a display of artefacts, but also of ideas and ambitions. If science exists with such powerful resonances in traditional Islam, the unstated message read, what could it say to the West today?

For generations, it has been the received wisdom in our schools that the Muslim world had, through the Dark Ages in Europe, even from the end of Alexandria to the rise of the Renaissance, acted as a cultural "carrier" — a custodian of the Graeco-Roman scientific tradition. Western eyes, closed to Islam after the fall of Constantinople in 1453, looked instead to passages and conquests overseas, eventually bringing the fruits of exploration and empire in tribute to a Europe emboldened by the "new philosophy" of the scientific revolution. In our schools we averted our eyes from Islam, portrayed as an ossified metaphysics, unproductive in the age of men committed to "the effecting of all things possible".

Yet, if one enquired, the position was never so simple. Surely the sources of discovery in Islam drew from the same wells as those which revived the "West"? Surely no historian could ignore the fundamental contributions to mathematics and medicine, optics and astronomy, pharmacy and biology, engineering and anatomy, associated with the Islamic world from North Africa to India? No student of Sarton or Miéll could ignore the discoveries of Ibn al-Haitham (Alhazen), Avicenna, al-Bīrūnī, or even Ibn al-Nafīs, even if, in our pages, where ideas count for more than men, they hovered like restless shadows between the heat and cold of the desert, concealed by enveloping gardens of mystic lore. Still, Islam, as a philosophical system, had its specialists and as such could be left to one side. Language and mystery silenced our doubts; our ignorance flourished.

Within the past 15 years, several factors have helped to alter this dismal prospect. First, there came the weight of fresh scholarship, notably that of David King, David Pingree and G.S. Kennedy, and the late Willy Hartner, paralleling and complementing Joseph Needham's work on China and A. Rahman's on India. This compelled European and American attention to long-neglected sources, to evidences of scientific

achievement associated with what after the seventh century AD became the Muslim world. In the process, the "Middle Ages" acquired a totally new connotation, as did our sense of "science" as a unique feature of Western civilization. As this process was at work among scholars, there came what appeared to be a renaissance of an "Islamic cultural nationalism", a phenomenon particularly noticeable in Pakistan and Iran (and one of which the Science Museum was an indirect beneficiary). That renewed interest, coupled with the oil crisis of the mid-1970s, lifted scholars with no previous experience of the Middle East or Central Asia into asking fresh questions about Islam, its political sects, its Qur'anic precepts and its conceptual and methodological legacy.

The values implicit in the legacy have now become a central subject of great importance to the West. Through the 1970s, with increasing international concern for the conservation of the global environment, came a "crisis in science", in large measure reflecting disillusionment with the politics of Western technology. This in turn bred sustained reflection and recrimination among historians and other scholars. For three centuries, the gift of Western science, the "touch of Midas", had held promise of eliminating poverty and materially developing the world; in the process, perhaps scientific solutions would eventually be found to eliminate even the boundaries and burdens of ideology. Alas, however, as the experience of the world revealed, the eutetic gifts of Midas had serious shortcomings. Western science, with its powerful belief in objective, value-free knowledge, taught us that what can be discovered must be discovered, without teaching us at what cost. Western technology had, as its basis, a categorical imperative — that everything which can be done will be done. But we were unprepared for the compromise in values this sometimes entailed. With our triumphs, now competed perils.

If the "Occident" was thus caught in a dilemma of its own making, what of Islam? Here, the question was not trivially one of cultural chauvinism, or the search for scientific priorities. The Muslim world had felt the force of the West's scientific and industrial revolutions, and now felt the weight of technological dependence. Any clash of values experienced in the West would be multiplied in the world of Islam. Where would lie the way forward, between value systems long at daggers drawn?

It is to this question that Ziauddin Sardar addresses himself in this first major publication of a programme of symposia on "Islam and the West". In conferences during 1981 and 1982, sponsored by UNESCO and IFIAS, and Islam and the West International, Sardar and 13 contributors confronted Islamic and Western approaches to science and explored answers to two questions. First, can in Islam be found a new synthesis, capable of offering Occidental rationality a humane vision of knowledge, to the benefit of all mankind? Second, can there be a distinct "Islamic science" which in some way can help reunify knowledge and responsibility?

If there is a right answer to either question, the key lies in understanding the relation between Islamic principles, precepts and practice. As M. Husain Sadr and others explain, Islam is a total epistemological system; there is no dichotomy between philosophy and science, economics and politics, or religion and society. As a holistic system, it permeates all human activity. There is, therefore, no distinction between the ends and means of knowing, and between knowing and applying that knowledge. Islamic teaching incorporates a framework of concepts — including the unity of God, trusteeship, worship, civilization, social justice and public interest. These concepts, if properly acted upon, will surely promote both social and ethical accountability. In pursuit of mutual understanding, participants explored Western and Islamic values and perspectives, debated interpretations of history, and grappled with the task of creating a common agenda.

There can be no doubt of the interest generated by their discussion, or of its importance in bridging discourses long distinct. What emerged is a shared view that ethical insights can and must be sought from Islam. But there are difficulties, as all participants recognized. For a start, as J.R. Ravetz asked, can we be certain about the problem? What, precisely, is the "crisis of science"? Is it a singularly Western phenomenon? If so, surely the ethical dilemmas of Western science must be faced within the Western tradition? Is science, as knowledge, in itself good, requiring only a renewed piety to qualify abuses and bring it to humane fulfilment? Or is science fundamentally a secular activity, owing allegiance to its own self-regulating criteria, its paymasters, and the interests and values of its practitioners? Further, what precisely is the range of these "values"? Is, as Munawar Anees would argue, God the source of all that is "valuable"? If so, in what sense are human values subjective? The bewildering complexity of such theological conundra threatens even the willing traveller.

For some, it may be that to travel hopefully is more important than to arrive. But a destination of a kind comes into view in the applications of the new "Islamic im-

perative" to the built environment. Essays by Lloyd Timberlake, Alison Ravetz, S. Parvez Munzoor and S. Gulzar Haider help both to articulate the environmental ethics of Islam, and give them practical expression. Haider's model of "habitat and values" traces architectural designs in houses and cities that embody precisely those principles of integrity, purpose and clarity which have for centuries fascinated Western travellers to the Middle East. Embracing metaphors of time and structure, he offers a bold synthesis in sand and stone.

Unavoidably, as Robert Walgate summarized, ethical exhortation can appear exceedingly nebulous when one is confronted by practical issues — from militant Islamic nationalism, to the eager acquisition of Western technology apparently regardless of what would be in the West understood as "public interest". What *can* Islam offer Western science? The jury is still undecided. The ethical debates of Western science during the past two decades have not penetrated Islam. Under the circumstances, what are the implications, ethical or economic, of the "Islamization" of science and technology, as espoused here by Ali Kettani? The faith has different aspects, revealing themselves sometimes as fundamentalism, sometimes as mysticism

and sometimes worldly-wise pragmatism. Which is to form our new view of science in Islam? Presumably, Islamic science must, in a practical sense, "work". We seek operational knowledge. How do we obtain it? Can there really be no sociobiology in Islam? And what of technology — can there be an Islamic bulldozer, and, if so, how would its construction or function differ from Massey-Ferguson's finest? Finally, what are the political dimensions of Islamic science? As Robert Walgate reflected, the cult of the expert gives great power in Islam to the *Ulamā*, just as the cult of expertise is given great power (and accountability) in the West. If one wants gold, can one escape the fate of Midas?

Mr Sardar would not claim these questions have been fully answered. The discussion must go on, East and West, aided by such promising sponsorship. From it arises a challenge for Muslim scholars to find ways of encouraging the translation of beneficent principles into practical action, whether in fashioning the human environment, directing the goals of medical research, or in the conduct of everyday life. As such, the task is clearly not confined to Islam. □

Roy MacLeod is Professor of History at the University of Sydney.

Biology of paradise

Mark Williamson

Ecology and Biogeography in Sri Lanka.

Edited by C.H. Fernando.

Dr W. Junk: 1984. Pp.505. Dfl. 265, \$110, £67.50.

Biogeography and Ecology of the Seychelles Islands.

Edited by D.R. Stoddart.

Dr W. Junk: 1984. Pp.691. Dfl. 300, \$115, £76.50.

BOTH Sri Lanka and the Seychelles have claims to be paradise on Earth. Both also have much to offer to biogeographers and ecologists, are tropical and are in the Indian Ocean. But while Sri Lanka is a large continental island (65,000 km²) with high mountains (up to 2,500 m), some rain-forest and a long history of cultivation, the Seychelles are minute (a total land area of only some 400 km²), in the middle of the ocean and include low coral islands, "high" limestone islands up to 8 m (of which Aldabra is the best known) and the uniquely isolated high granitic islands, rising to 900 m.

These two volumes, the latest in the *Monographiae Biologicae* series, continue a tradition of discussing interesting islands. Past volumes have covered the Canaries, New Guinea, Newfoundland and the Pityusics (Balearics). However, the intent of the series is not clear to me. The

monographs are advertised as "hydro-biology", and most deal with rivers and lakes, but a case study of an ancient man-made lake in Sri Lanka (there are no natural lakes) comes in the *Developments in Hydrobiology* series from the same publisher.

So what features of island life would one expect to be covered in such a monograph? Something on geology, certainly, and something on climates. Islands have interesting relic and endemic species, so there should be something on their origin and, by now, on the rate of evolution. Since Darwin, biologists have been interested in dispersal to islands, a topic often contrasted nowadays with vicariance biogeography. The number of species, their population sizes, their turnover by immigration and extinction, the representation of this in species-area curves, and by incidence and prevalence curves, are all topics of current interest. Questions of adaptation and life history strategy naturally arise from all this, and indeed islands are generally regarded as useful experiments. But not as yet, it would seem, in the Indian Ocean.

This is not to say that some of these topics are not dealt with, and indeed dealt with quite well, in the Seychelles volume, and some of them are touched upon in the volume on Sri Lanka. But the difficulties seem to be two. The first — and this applies particularly to Sri Lanka — is that taxonomy is still fairly primitive, even in groups like mammals. But the major problem is the complexity of geological history in the Indian Ocean. During the Tertiary it re-

sembled a dodgem stand at a fair — land whizzing in all directions, though with a pattern discernible overall; collisions here, pieces stuck there. As Braithwaite says, "if displacements in the Atlantic had been so elaborate the verification of concepts of sea-floor spreading might have been delayed for years". While the authors in the Seychelles volume do their best to get to grips with the geological background, those in the Sri Lankan volume seem mostly unaware of these phenomena, except for the occasional mention of Gondwanaland.

The volume on Sri Lanka is not so much a monograph, more a scrapbook. Interesting topics crop up, but there is little coherence. The worst chapter is "Freshwater Invertebrates, Some Comments", which comprises just two and a half pages of references to previous works. There is an account of grasslands, but not of rain-forest. There are chapters on the ecology of rice fields, on Monogenea from freshwater fishes, on coastal lagoons, on parasites of the endemic and relic vertebrates, but the only vertebrate group dealt with is mammals. There is emphasis on freshwater habitats and organisms, and also on the human aspects, such as malaria, man-made lakes and land use. It is difficult to steer a course between a superficial account and a tedious catalogue, but this volume too often hits the rocks on both sides.

In contrast, the volume on Seychelles is comprehensive and stimulating, though one weakness is that the information on, say, the areas of different islands is scattered through various chapters. There are accounts of the geology and climate, coral reefs and the marine fauna, vegetation and floristics, land molluscs, terrestrial arthropods, herpetofauna, birds and on man's influence. The high Seychelles are the only small granitic islands in the middle of an ocean, and they have a remarkable fauna and flora to suit. The absence of amphibians comes into the standard definition of an oceanic island, but on the Seychelles there are five species of frogs and seven species of caecilians, burrowing legless forms; the latter at least Gondwanan relics. An introductory historical chapter includes good maps, a table of diversity and endemism, and species-area curves.

It is really rather odd to find two volumes in the same series, appearing at the same time, which differ so much in quality. For while the monograph on Sri Lanka will be of short-lived interest, even to specialists, that on the Seychelles may well be a standard reference work for many years. □

Mark Williamson is Professor of Biology at the University of York.

● Also published by Dr W. Junk in the series *Monographiae Biologicae*, volume 56, is *The Amazon: Limnology and Landscape Ecology of a Mighty Tropical River and its Basin*, edited by Harald Sioli. The series editor is H.J. Dumont and the price is Dfl. 450, \$172, £114.50.

Around the world in eighty years

Harriet Guest

Voyage into Substance: Art, Science, Nature and the Illustrated Travel Account, 1760-1840.

By Barbara Maria Stafford.
MIT Press: 1984. Pp.645. \$39.95,
£41.75.

FROM its foundation in 1660 the Royal Society was aware of the capital value of the "Philosophical stock" accumulated by "Voyages into all parts of the World". Following the invention of a reliable marine chronometer, the later eighteenth century saw a proliferation of voyages (and land expeditions) from Europe, one of whose aims was the garnering of further philosophical stores. This aspect of exploration and travel accounts as investigations and surveys of nature forms the subject of Professor Stafford's *Voyage into Substance*.

The book is, as its title suggests, a journey into a considerable range of subject matter: Professor Stafford discusses and refers to a mass of accounts, and an almost overwhelming variety of locations, ranging from the Sahara to the Arctic, from the microscopic to the planetary, from the mine shaft to the balloon. Almost inevitably, however, this vast range poses problems of organization and method. The author argues that the diverse representations of nature that fall within the scope of her study are unified as expressions of the "scientific gaze". This gaze is achieved through a willed act of seeing, which somehow voluntarily denudes itself of cultural content or accretions, as "scientific scrutiny recaptured the innocent eye of the archetypal encounter with the earth". She claims that "scientific perspicacity... inaugurated a new visual habit of plainness or real transparency", and disclosed a nature which declared its own history and meaning. This innocent vision finds its

expression in "a verbal (and pictorial) scientific style", of "masculine" plainness and accurate particularity, where "Art as scientific language, as limpid and democratic medium for the transcription of ipseity, does not self-expressively alter the pure presence of the properties it remands". It was evidently a style very different from Professor Stafford's.

The extent to which the procedures, at least, of the scientific enterprise were involved in and informed by its cultural contexts may be obscured by Professor Stafford's very selective notion of natural philosophy. The Royal Society, in its *Secret Instructions* to Cook, enjoined him to observe and collect a considerable variety of inanimate natural phenomena, but it also instructed him to "observe the Genius, Temper, Disposition and Number" of the native peoples he encountered. A large proportion of the 270 illustrations to this book carry out the same instruction: when, for example, William Hamilton illustrated the eruption of Vesuvius, he included in his aquatints some Neapolitan spectators of the event, and their expressive figures

clearly invite us to see it as more than an object of scientific scrutiny. But Professor Stafford comments only on the "probing scrutiny" of the artist's gaze, and indeed almost never refers to the interpretative functions of human figures and compositional structures in landscapes. She quotes Georg Forster's intention "to throw more light upon the nature of the human mind, and to lift the soul into that exalted station, from whence the extensive view must justify the ways of God to man...", and its triple literary allusion to Pope, Johnson and Milton indicates that the aims of his account are not purely those of science as she has defined it, and that his language is neither plain nor transparent.

For Professor Stafford, science is the "recording of phenomena stripped of human history". This may have been the aim of some travellers and of most explorers, but it can hardly be said that they achieved it, unless, like Professor Stafford, we take the will for the deed. □

Harriet Guest is a Research Fellow at King's College, Cambridge.

Separating out

C.S.G. Phillips

Contemporary Practice of Chromatography.

By Colin F. Poole and Sheila A. Schuette.

Elsevier: 1984. Pp.708. Dfl.159, \$61.25.

THIS book is, in effect, a highly complex and competent review of the present state of the art in the techniques of gas chromatography, high-performance liquid chromatography and high-performance thin-layer chromatography. Little space is wasted on the history of chromatography, on the wide variety of chromatographic methods that have been devised but are now infrequently used, or on the applications of chromatography other than for

analysis or preparative work. There is no reference to the various abstracting services available, or any general guide to the literature, while the wide use of computers with chromatographic instruments is only touched upon.

Where the book excels is in its attention to all the particular practical details of operating modern efficient chromatographic systems. It contains a mass of useful and critically considered information, backed by extensive bibliographies. There are two long, extremely helpful and illuminating chapters on the preparation of samples for chromatography and on "hyphenated" methods (such as mass spectrometry, Fourier transform infrared and nuclear magnetic resonance) for the identification of components after they have been separated by the chromatographic column. *Contemporary Practice of Chromatography* will surely prove to be a most valued guide and source of reference for all practising chromatographers.

The beginner, however, will not find it an easy book to read; for all its many virtues, it will not serve as a general introduction to the subject. The reader is expected to know too much, technical terms are frequently used without explanation, and even when explanations are provided they are sometimes difficult to find and to follow (the index is quite inadequate). Further, the general introduction is not always convincing: for example, on page 31 alone I found four statements with which I would take issue. So although this book provides a mine of information, clearly it is one where some hard digging is required. □

C.S.G. Phillips is a Lecturer in the Department of Inorganic Chemistry, University of Oxford.



View from above — the Battle of Fleurus, 1793-94. Taken from *Voyage into Substance*.

Chemical bondage

Geoffrey Wilkinson

Dictionary of Organometallic Compounds, Vols 1, 2 and 3.

Executive editor J. Buckingham.
Chapman & Hall/Methuen: 1984.
Pp. 3,000. £495, \$990.

ALTHOUGH some organoarsenic compounds were known previously, organometallic chemistry, in which organic molecules or radicals are bound to metallic elements by metal-carbon bonds, really originated with two major discoveries in the nineteenth century. In 1827, in Copenhagen, Zeise discovered the salt $K[PtCl_2(H_2O)_4]$ whose constitution as an ethylene complex, the first of the so-called "π complexes", was not fully established until the 1950s. Then in 1845, at a school in Hampshire, Frankland discovered the first true alkyl, diethylzinc. This discovery not only revealed a new area of chemistry but contributed substantially to the concept of valency. The later discovery of magnesium reagents by Grignard opened up even more possibilities as these were easier to handle as synthetic reagents than the flammable zinc compounds. The third major class is the carbon monoxide compounds, discovered by Schutzenberger and by Mond. Although "carbonyls" are not, by strict definition, organometallic, they have metal-to-carbon bonds and carbon monoxide chemistry is now so interlinked with that of the alkyls, aryls, olefins, acetylenes and related or derived compounds that it has become usual to include carbonyls under the general title.

Organometallic chemistry thus includes all manner of compounds, many of which are extremely unusual both in their structure and bonding. During the first half of this century the chemistry was dominated by organocompounds of the main elements — lithium, magnesium, mercury, silicon, phosphorus, arsenic and so on — and by utilization of magnesium and lithium alkyls and aryls in organic synthesis. Transition-metal organometallic chemistry developed only after about 1952. Carbonyl chemistry proceeded essentially independently, mostly in pre-war Germany through the work of Hieber and his school and of Fischer, Tropsch, Roelen, Reppe and others in industry where the use of transition metal compounds led to the development of new syntheses and to new industrial processes.

Over the past 35 years these two major streams have, to a large extent, merged, and organometallic chemistry today is a major field with its own journals, works of reference and textbooks. The number of papers and patents published annually has risen from a handful to thousands.

This *Dictionary* provides a list that comprises "those compounds which are in the opinion of the Specialist Editors, most

typical, representative, interesting and useful or potentially useful and [presents] their properties and selected bibliography in an orderly and systematically indexed fashion". Quite a mouthful. The elements are treated in alphabetical order from silver (Ag) to zirconium. Each entry includes details of stoichiometry and molecular weight, and of some simple properties such as form, solubility, thermal and air stability, and toxicity, together with a few references to synthesis and spectra. The code-numbered compounds are also given their systematic name which many may find useful; for some of the more complicated compounds, trying to work out the name is often a substantial challenge and far more difficult than in organic chemistry. Structural diagrams are commonly provided and a useful feature is that these are repeated in the summary list at the beginning of each section. The index volume contains full names, molecular formulae and CAS registration numbers.

For workers in organometallic chemistry

and catalysis, in both academic and industrial laboratories, this mammoth work will be very useful. It may be regarded as the Red Bible to supplement the now well-known Green Bible, otherwise the nine-volume *Comprehensive Organometallic Chemistry* published by Pergamon in 1982. The latter has discussions of various sorts in the descriptive text with copious data tables, while the *Dictionary* is designed to allow rapid access to limited synthetic and structural information. Readers may notice omissions; thus, out of the 145 manganese compounds no simple alkyl, such as $Mn(norbornyl)_4$ or $[Mn(CH_2SiMe_3)_2]_n$ is listed. Nevertheless, the editors' choice is pretty extensive and doubtless the promised annual supplements will fill in gaps as well as provide extensions. Expensive though it is, many institutions will have to acquire this work. □

Geoffrey Wilkinson is Sir Edward Frankland Professor of Inorganic Chemistry at Imperial College, University of London.

Starry night

Carole Stott

Maps of the Heavens.

By George Sergeant Snyder.
André Deutsch, London, Abbeville,
New York: 1984. Pp. 144. £19.95, \$45.

THE game of joining the dots is not new. The Babylonians had such a pastime. They drew pictures by joining dots of light, or rather stars, in the sky. This ancient tradition was followed by Ptolemy whose *Almagest* (circa AD 150) lists over a thousand stars, grouped into 48 pictures or constellations. Today the sky is divided into an internationally accepted system of 88 constellations of which the Ptolemaic group forms the core. Many of the very earliest artistic representations are lost forever, and it is only from the advent of the printing press in the sixteenth century that we have examples in abundance. The first printed star maps were Dürer's, of 1516, and in the 300 years following, artists, mathematicians, cartographers and astronomers have all set down their own interpretations of the dots in the sky.

In *Maps of the Heavens*, George Snyder, who is in charge of maps and cartographic materials at Sotheby's New York office, looks at the evolution of the iconography of constellations in these more recent maps. The familiar 48 creatures and figures of mythology — Pegasus, Andromeda, Perseus, a lion, a dragon, dogs and bears and the rest, all of which Snyder lists — were joined by new figures. The observational work of astronomers such as Tycho Brahe, the revelations of the telescope and voyages to the Southern Hemisphere added new stars ready for forming into constellations. The stars

became animals, religious figures, political symbols or tools of the sciences and arts. Some are still in use today; others, like La Lande's immortalization of his cat, Felis, or Schiller's replacement of the 12 signs of the zodiac by the 12 apostles, were short-lived.

Snyder's illustrations, which are predominantly from the sixteenth and seventeenth centuries, appear in almost chronological order. Many of them have been chosen for their beauty, not for their importance as stepping stones in the history of celestial cartography; the reader will search in vain for the influential Islamic constellations of Al Sufi, the popular and extensive *Uranographia* of Bode, and Piccolomini's star atlas of 1540 (which was not only the first printed star atlas but the first printed set of maps of the stars, as distinct from pictures of constellations). Rather, Snyder addresses himself to the colourful pageantry of celestial mapmaking.

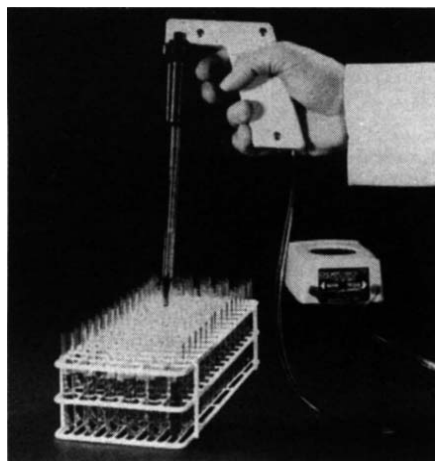
Many of the maps betray their date or cultural and religious background through the artist's adoption of a certain artistic tradition. Here, however, the difference between the Eastern- and Western-style constellations is only hinted at as all but a handful of the 75 illustrations are from European countries. The joy of the book is the colourful and realistic photographic reproduction of the maps, which will appeal to a scientific as well as the intended, more popular audience. Each illustration is captivating in itself, while the complementary text, placed adjacent to the map, is reminiscent of an extended and authoritative catalogue entry. Consequently the book can be started and finished at a minimum of 75 different points. □

Carole Stott is Curator of Astronomy at the Old Royal Observatory at Greenwich, London, part of the National Maritime Museum.

New gadgets for the lab

Recent additions to the range of instruments available for the biochemical laboratory include a sophisticated rotary mixer and an automation system.

● A new mixer from Coulter Electronics, the Rotary Coulter Mixer, will accommodate and mix up to 32 samples per tray, with the option of additional tray assemblies that can be pre-loaded. The mixer rotates samples at an angle of 45, 60 or 75 degrees, ensuring flexibility, whatever tube size is used. A mixing angle of 45 degrees is recommended for the commonly used 5 and 7 ml tube sizes; larger diameter containers may be more efficiently mixed using 60 or 75 degree end-over-end. The Coulter Mixer provides an efficient mixing process of end over-end inversion and accepts all standard tube types. *Circle No. 100 on Reader Service Card.*



Automated pipetting by Drummond.

● Drummond Scientific Company has introduced an improved, inexpensive nosepiece modification kit for the Drummond Pipet-Aid. The kit incorporates a dual hydrophilic/hydrophobic filter which is designed to prevent over-pipetting and cross-contamination. The new Tissue Culture Failsafe Nosepiece is available as a retrofit kit and will also be available as an option for new Drummond Pipet-Aids. Each kit contains all necessary parts including four filters. *Circle No. 101 on Reader Service Card.*

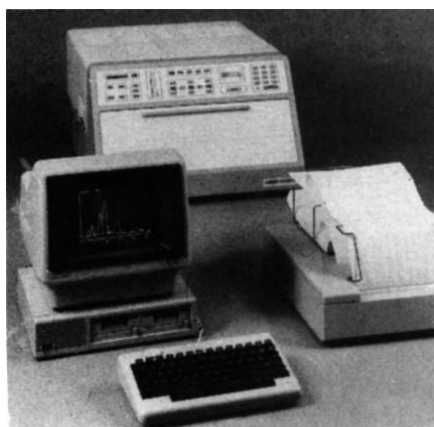
● A new storage box from Boehringer Mannheim provides a useful way of storing molecular biology enzymes. The strong plastic box is fitted with a polystyrene insert with space for 50 microcentrifuge tubes. The boxes provide a convenient means of storing enzyme stocks, buffers and DNAs at temperatures down to -80°C . *Circle No. 102 on Reader Service Card.*

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● The Z100A Zymate laboratory automation system, from Zymark Corporation, combines microprocessor-controlled laboratory robotics and work stations with dedicated engineering support. The Zymate system automates laboratory procedures involving steps such as weighing, liquid-handling, filtration, centrifugation, liquid-liquid and solid-phase extraction and sample conditioning (mixing, heating, cooling and evaporation). The system provides automated sample preparation for laboratories performing titrations, BOD measurements, XRF, ICP and AA, GC HPLC and NMR. It can be directly interfaced to these and other analytical instruments or can be used to fill racks or carousels with prepared samples. *Circle No. 103 on Reader Service Card.*

● DNA synthesis support columns for Applied Biosystems Model 380A DNA synthesizers are now available from American BioNuclear. Endcaps and columns are precision machined from inert plastic to give leak-free performance, and retaining filters are constructed from Teflon mesh allowing maximum flow rate without loss of support. Aluminium crimp seals are secured by hand and each column is inspected before shipment. The column packing material is derivatized long-chain alkylamine controlled pore glass (LCAA-CPG), prepared to a loading of 20–30 μmol per gram. *Circle No. 104 on Reader Service Card.*

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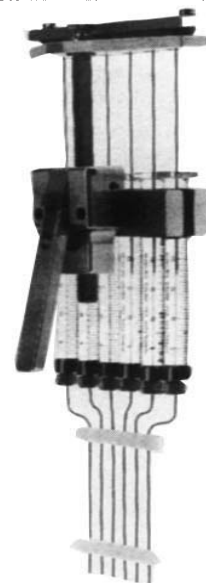


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● Hamilton Company's new line of Terasaki/Microtitration dispensers delivers six equal liquid volumes simultaneously and repeatably, from 0.5 μl to 10.0 μl . Dispensers are available with needle spacings of 6.35 mm for Terasaki/HLA plates, or 9 mm for standard microtitration plates. Each dispenser consists of six identical Hamilton Gastight/Microliter syringes, mounted in a special clamping system. When the lever is depressed, all six syringes are advanced simultaneously, dispensing 1/50th of the syringe volumes. This way, aliquots can be dispensed 50 times without refilling. *Circle No. 107 on Reader Service Card.*

● Philips' latest microprocessor-controlled ion-selective analyser, the compact PW 9415 ion-selective meter, offers direct measurement of mV, pH, pX, degrees centigrade and concentration, together with the incremental methods of standard addition and subtraction, and sample addition and subtraction. A special low-level concentration mode helps the operator to obtain results even when the electrode has a nonlinear response. The operator is taken through the measurement sequence with an easily recognized series of prompts. Results are shown on the display and may be re-processed on a printer or computer if required. Other standard features of the PW



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How to avoid the 19 critical mistakes in buying fermentation equipment

Make one mistake and you can have problems that include inadequate process control, permanent operator inconvenience and costly downtime.

Now NBS has published a comprehensive review of the problems and pitfalls that await the unwary buyer of industrial fermentors — and some very practical suggestions to help avoid them.

Called *19 Critical Mistakes Made in Buying Fermentation Equipment and What You Can Do to Avoid Them*, this practical guide is available without charge.

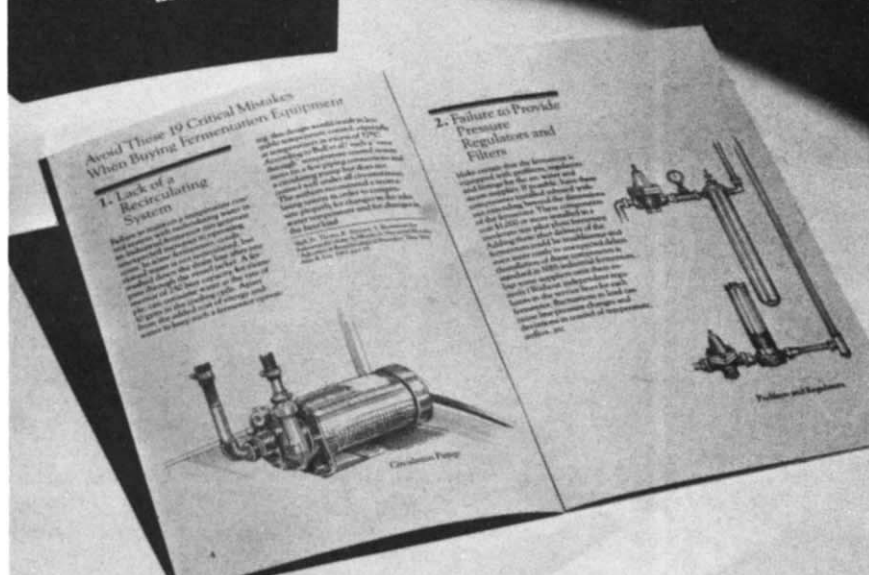
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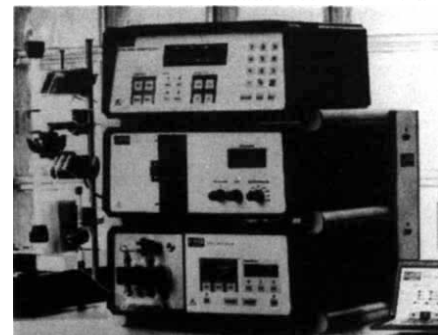
**19
CRITICAL
MISTAKES**
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fermentation
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and
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can do to
avoid
them



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● The Heraeus-Vötsch VLK 07/90 Labotest is a new compact cabinet for humidity and temperature tests specially designed to take up a minimum of space, and as it is fitted with castors it is also extremely manoeuvrable. It offers a temperature range of -70°C to 180°C by means of a built-in cascade refrigeration system. The large non-reflective glass door gives a clear view of the inner working space, which is illuminated to make it easy for the operator to observe specimens under test. The Labotest has a wide performance range, with continuous operation possible up to 85°C and 85% relative humidity, and it satisfies most international standards.

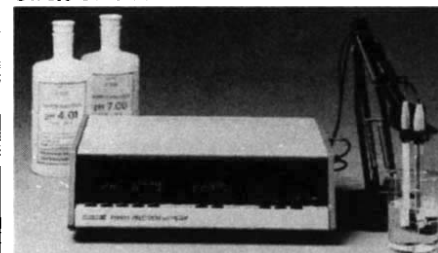
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Automated chromatography—the new LKB Ultrochrom.

● LKB's UltroChrom GTi system offers multimode separation of macromolecules, through a system combining the best features of LPLC, HPLC and FPLC. Designed for the biochemist working with proteins, peptides, nucleic acids and even carbohydrates, GTi provides fast separation, high resolution and increased bioactivity. The biological activity of proteins after purification is generally between 91% and 99%. By using gel filtration (GFC), ion exchange (IEC) and hydrophobic reaction chromatography (HIC) in one system, UltroChrom GTi makes it simple to select the most appropriate chromatography for any problem. The system offers true HPLC, so can be used for mainstream reversed phase chromatography, including microbore and LC.

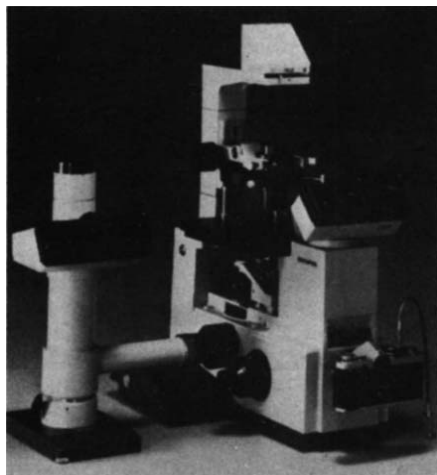
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The Radiometer PHM85.

● The Radiometer PHM85 precision pH meter is designed for high-precision measurement of pH, pX, mV and temperature. The resolution is 0.001 pH, 0.001 pX, 0.1 mV and 0.1°C . The buffer values can be selected as desired and are easily keyed-in and stored in the microprocessor's memory.

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The Olympus IMT inverted microscope, suitable for brightfield, phase contrast, Nomarski differential interference contrast, reflected light fluorescence, cine/photomicrography and micromanipulation. Circle 112 for full details.

● Delivery systems in which up to four solvents may be blended can be used in the analysis of a wider range of samples than two or three solvent systems. This versatility is characteristic of Perkin-Elmer's four-solvent delivery systems for higher performance liquid chromatography, the proven Series 4 and the new Series 400. The new system can be used for all types of HPLC — microbore, analytical, high-speed and semi-preparative, and dedicated single-function command keys simplify operation. Each 'method' consists of up to ten programming steps and nine user-defined methods can be stored. Low conversion volumes allow solvent compositions to be changed in seconds, so that changing from one method is simple to another. The Series 400 pump can be programmed for isocratic or gradient analysis with up to four solvents. Simultaneous solvent and flow programming is standard.

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The Series 400 liquid chromatograph from Perkin-Elmer.

● A new system from Forma Scientific helps to increase anchorage dependent cell yields in microcarrier applications. Micro-Stir magnetic stirrers with Magna-Flex flasks gently mix microcarrier beads at slow speeds, so that anchorage-dependent cells are protected, helping to increase product yields. The four-position stirrer features adjustable speed control plus stirring and delay timers for precise, repeatable cycles. A single-position unit with speed control only is also available. Both units easily fit into Forma incubators for culturing under controlled conditions. Flasks in 500 and 1,000 ml are available.

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Microcarrier culture in the Forma Magna-Flex.

● In high resolution micromanipulation given orthogonal mechanics of the highest fidelity, the operator is the limiting factor in attaining increments of the order of 0.10 μ m. A new micromanipulator from Hacker Instruments overcomes this problem with push-button control. After completing coarse and fine micrometre positioning, the operator selects the direction or translation and increment on the control unit and initiates movement at the push of a button.

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● Hi-Tech Scientific's QF-10 preparative quencher is microprocessor controlled and facilitates the chemical and/or freeze quenching of a fast chemical reaction after a known ageing time. This makes it possible for a fast reaction to be studied by an intrinsically slow technique such as gas-liquid chromatography, or radio isotope counting. The ageing period is continuously variable from 5ms to 10.3s.

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● Amersham International's new Hybond blotting membranes can be used for immobilization and analysis of nucleic acids and proteins, enabling transfer of separated bands or spots from electrophoretic gels. The membranes come in a range of sizes and are available in either nitrocellulose or supported nylon-66. With each pack is an introduction and guide to blotting techniques with basic protocols and notes on adapting these to specific requirements.

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● The Thermex-IBM thermometer interface, available from Columbus Instruments, converts popular IBM-PC or XT computers into a 16-channel printing thermometer with disk data storage capacity. Thermex provides 16 individual channels for 16 thermocouple probes with cold junction compensation provided for each individual probe. Columbus provides a complete hardware/software package plus a variety of temperature probes calibrated in the range 0 to 50°C with accuracy of 0.1°C. Thermex can be used in any other temperature range with a special probe calibration program. Biological temperature measuring probes are available from Columbus for rectal, intravenous and implantable applications.

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● The Belgian company Consort pvba has developed a new microcomputer electrophoresis power supply equipped with three different programs in a memory to control the whole separation procedure. Using this technique, some separations can be run automatically overnight without risk. The supply is available in two versions, each with a maximum power of 200 W. Model E614 gives up to 1,000 V/400 mA and model E632 up to 3,000 V/150 mA.

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● A 12-page brochure from Gilson describes the company's HPLC system software with combined gradient control and data analysis functions. The new Data Master software package provides a wide range of analysis functions, including real-time and post-run quantification of peaks. The package is included with the System 45 gradient analytical and the System 55 preparative chromatographs. Both use the Apple IIe computer as system controller.

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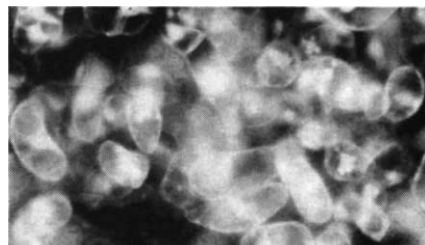
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New plant tissue culture collection

THE American Type Culture Collection has recently established a plant tissue culture bank. The collection will accept well-documented plant tissue cultures with properties useful in research, teaching, or industry. These cultures will be made available to the scientific community for a fee to cover expenses. Cultures are also accepted for patent application purposes. At its opening last December there were 62 cultures in the bank including alfalfa, blueberry, birdsfoot trefoil, carrot, celery, corn, datura, lily, rice, soybean, sugar



ATCC 54009, a fluorescein diacetate stain of *Apium graveolus*.

cane, tobacco, tomato, walnut, wheat, and tumour cell lines of sunflower and grapes induced by *Agrobacterium tumefaciens* carrying Ti plasmids.

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THE NO-SHAKE SHAKER



This heavy-duty, large-capacity tier shaker shakes large numbers of flasks—not your lab. With a full load of 432 flasks, it runs smoothly and quietly, yet never needs to be bolted to the floor. Run it at speeds up to 300 rpm and it won't budge or creep year-after-year. A solid state electronic controller gives precise and repeatable speed settings.

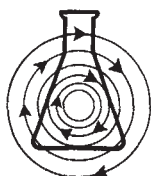
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● The latest compact Micro-Stat analysers for glucose, urea, cholesterol, lactate and other metabolites from Analox Instruments provide results in 20 seconds from only 10 microlitres of sample. All models are fully computerized with keyboard and word display. An integral printer includes statistical functions for performance assessment with quality control materials. The GM7 and LM3 analysers use an electrochemical sensor detecting oxygen change or rate-of-change in oxidase/substrate reactions. Coupled dehydrogenase-oxidase reactions greatly extend the range of potential analates. The LM3 will determine all the oxidase substrates of the GM7 and dehydrogenase coupled analates, such as lactate.

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Dataplate accurately controls both temperature and time.

● The Whatman Dataplate controller is a new microprocessor accessory for temperature and time control. To use the controller, the selected hotplate or heating equipment is plugged into the controller's socket. The temperature probe is placed in the sample (or oven thermometer receptacle) and the required temperature and heating time instructions are keyed in. From that moment the Dataplate controller monitors the process continuously by microprocessor-controlled pulsed power input to the heater until the automatic cut-off terminates the process.

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The personal film badge, part of the ICN monitoring service.

● ICN Biomedicals' personal dosimetry services for US laboratories include: a film badge for each individual, monthly or quarterly computer analysis, legal proof of state and federal regulations, and emergency reporting in case of excessive radiation exposure. Laboratories that participate also receive an annual summary report.

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